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(54) Title: GENE EXPRESSION PROFILES IN LIVER DISEASE

(57) Abstract: The present invention results from the examination of tissue from hepatic carcinomas to identify genes differentially expressed between cancerous liver tissue and diseased but non-cancerous liver tissue. The invention includes diagnostic, screening, drug design and therapeutic methods using these genes, as well as solid supports comprising oligonucleotide arrays that are complementary to or hybridize to the differentially expressed genes.



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## GENE EXPRESSION PROFILES IN LIVER DISEASE

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### RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Applications 60/341,815 and 60/343,185, both of which are herein incorporated by reference in their entirety.

5

### FIELD OF THE INVENTION

The invention relates generally to the changes in gene expression in liver tissue from patients with hepatic carcinomas. The invention specifically relates to a set of human genes that are differentially expressed in cancerous liver tissue compared to  
10 diseased, but non-cancerous liver tissue.

### BACKGROUND OF THE INVENTION

#### Liver Disease

Generally, liver disease is classified as a disorder that causes the liver to  
15 malfunction or cease functioning all together. Cirrhosis, for example, is a group of chronic liver diseases in which liver cells are damaged and then replaced with scar tissue, thereby decreasing the amount of normal liver tissue. While it is most often caused by alcohol abuse, patients with hepatitis infections and other biliary diseases can also develop cirrhosis. Chronic hepatitis-B infection, hepatitis-C infection, and cirrhosis have all been  
20 shown to have strong associations with primary liver cancer, although the mechanisms involved are still not fully understood (Wu *et al.* (2001), *Oncogene*, 20: 3674-3682). About 10-20% chronic hepatitis-B infections result in primary liver cancer. Other factors such as alcohol consumption, poor nutrition and aflatoxins (carcinogens produced by molds, which are found in spoiled foods such as peanuts, corn, grains and seeds) are also  
25 linked to the development of primary liver cancer and cirrhosis.

In primary liver cancer, liver cells become abnormal, grow out of control and form malignant tumors. This disease is also called hepatocellular carcinoma (HCC) or malignant hepatoma. Cancer that spreads to the liver from another part of the body as a result of metastasis is not the same disease. HCC is difficult to detect at an early stage  
30 because the symptoms are not specific. They include loss of appetite and weight, fever,

fatigue and weakness. As the cancer progresses, pain may develop in the upper abdomen, extending to the back and right shoulder. Swelling or a palpable mass may also be present in the upper abdomen, along with jaundice and darkened urine. When the cancer metastasizes, it typically targets the lungs and brain.

5       Diagnosis of HCC may be made by blood tests, in particular, tests for tumor markers such as alpha-fetoprotein. About 50-70% of HCC patients show elevated levels of alpha-fetoprotein. Additional diagnostic methods include non-radioactive imaging (abdominal or chest x-rays, angiograms, CT scans and MRIs), liver scans using radioactive materials and liver biopsies. Treatment of HCC is often not successful,  
10       because detection is often too late, but methods include surgical removal of the cancer, chemotherapy and radiation, alone or in combination. Although HCC is not very common in the United States, it is very prevalent in parts of Asia and Africa, largely due to the higher incidence of infection with hepatitis viruses (<http://cis.nci.nih.gov/>;  
[http://cancer.med.upenn.edu/disease/liver/intro\\_liver.html](http://cancer.med.upenn.edu/disease/liver/intro_liver.html)).

15       The number of new cases of acute and chronic viral hepatitis has been estimated at approximately 200,000 per year in the United States. The viruses that commonly cause hepatitis are hepatitis A, hepatitis B (which is also oncogenic), hepatitis D (or delta hepatitis, a "defective" RNA virus that is infective only in the presence of hepatitis B virus), hepatitis C, hepatitis E (or epidemic non-A non-B hepatitis), hepatitis F and G  
20       (epidemic non-A non-B non-C variants which may be mutants of hepatitis B, but which do not express the B antigens), cytomegalovirus, Epstein-Barr virus and herpes simplex virus. The last three are prominent in patients receiving immunosuppressive treatments following liver, kidney or bone marrow transplants. Hepatitis viruses B, C and D are typically associated with chronic viral hepatitis.

25       Among hepatitis viruses, hepatitis B is associated with the greatest mortality. This virus is a double-shelled DNA virus with an endogenous DNA polymerase and a single, circular molecule of DNA 3200 base pairs in length. The virus replicates via an RNA intermediate requiring reverse transcriptase. In patients infected with hepatitis B, three types of particles can be detected in the serum, 20 nm spheres, tubules 20 nm in diameter  
30       and 100 nm in length, and complex, 42 nm Dane particles. Similar to the human hepatitis B virus are hepatitis B viruses found in ducks, herons, squirrels and woodchucks.

Diagnosis of hepatitis B is usually made by finding the surface antigen (HBsAg) in serum. The presence of HBsAg for six months or more signifies a carrier state or chronic infection. Anti-HBs (HBsAb) accounts for recovery and immunity.

Although the core antigen (HBcAg) is not detectable in the blood, the antibody can  
5 be detected. In cases of acute hepatitis, an IgM antibody to the core antigen (HBcAg) is found. But, if this antibody persists, it is an indication of chronic viral hepatitis. Another indication of chronic infection is a high level of IgG HBcAb, without HBsAb, but with HBsAg.

HBeAg correlates with active viral synthesis and infectivity. Appearance of the  
10 antibody (HBeAb) is a sign of reduced infectivity and that the patient will recover.

Only about 2-8% of adults infected with hepatitis B develop chronic infection, but about 90% of infected neonates become carriers. Chronic hepatitis B infection is associated with cirrhosis, as well as with liver carcinomas, as about 25% of these patients eventually develop cirrhosis. Although there is no universally effective treatment for  
15 either chronic or acute hepatitis B infection, current treatment methods involve administration of interferon alpha-2a, at dosages of, *e.g.*, 10 million units 3 times a week for 16 weeks, or 5 million units daily for 4 months.

Hepatitis C virus is considered to be the major cause of post-transfusion hepatitis. Another important source of infection is intravenous drug use. This virus is classified in  
20 the togavirus family of lipid envelope viruses, producing particles of 30-60 nm. It is a single-stranded RNA virus with a genome of about 10.5 kb. About 50% of patients infected with hepatitis C develop chronic infections, and about 20% of patients chronically infected develop cirrhosis. As mentioned above, it has been noted that many hepatitis C patients go on to develop liver cancer, but the percentage has not yet been established.  
25 Efforts at treating hepatitis C have been frustrated by the results that current antivirals, including alpha interferon, have not been very effective (<http://www.arenas.com/brian/viral.htm>).

Cirrhosis of the liver, typically caused by toxins, inflammation or metabolic disorders, is characterized by widespread nodules combined with fibrosis. Damaged or  
30 dead liver cells are replaced by fibrous scar tissue, which leads to fibrosis. Liver cells regenerate in an abnormal pattern, producing nodules surrounded by fibrous tissue. The fibrosis and nodule formation cause distortion and blockage of the liver's structural components, causing impaired blood flow and biochemical function.



In the circulatory system, blood from the intestines and spleen flows to the liver via the portal vein, before returning to the heart via the hepatic vein. Blood also flows directly to the liver from the hepatic artery. In the esophagus, stomach, small intestine and rectum, the body's systemic circulation is connected to the liver's portal circulation, and, under  
5 normal conditions, there is no backflow from the portal circulation into the systemic circulation. In cirrhosis, however, the fibrous scar tissue decreases blood flow to and through the liver. Blood then backs up in the portal vein and portal circulation, causing complications in other organs, such as enlargement of the spleen with sequestered blood cells, reduced platelet count and abnormal bleeding. Backflow of blood into the systemic  
10 circulation can cause varicose veins in the esophagus, stomach and rectum, which can rupture and bleed profusely. Hypertension in the portal circulation can also produce fluid accumulation in the abdomen (ascites) and surrounding tissue (peripheral edema), while decreased bilirubin secretion can lead to elevated levels of bilirubin in the blood and jaundice. Abnormal biochemical changes due to cirrhosis include decreased levels of  
15 albumin (which aggravates the ascites and edema), decreased levels of clotting factors, gynecomastia in men (impaired estrogen metabolism), and decreased metabolism of sugars, triglycerides and cholesterol. In advanced stages of cirrhosis, abnormalities in the brain can occur, because toxic substances normally removed by the liver flow to the brain. Changes include decreased mental function (concentration and cognitive abilities), stupor,  
20 coma, brain swelling and death.

In patients displaying some of the above symptoms, diagnosis of cirrhosis is usually easy, but cirrhosis may be difficult to detect in its early stages. Subtle changes occurring in the early stages include red palms, red spots on the upper body that blanch, hypertrophy of the parotid glands, fibrosis of the tendons in the palms and gynecomastia.  
25 X-rays and radioactive tracer tests may be effective, but diagnosis must often be by liver biopsy.

The structural damage to the liver is irreversible, although the underlying causes may be treated to stop progression of the disease (alcoholism in particular). The sequelae of the disease may also be treated (such varicose veins, ascites and edema). In alcoholics  
30 who have stopped drinking for extended periods of time, liver transplants have been successful (<http://cpmcnet.columbia.edu/dept/gi/cirrhosis.html>).

#### Molecular Changes in Liver Disease

Little is known about the molecular changes in liver cells associated with the development and progression of liver disease. Accordingly, there exists a need for the investigation of the changes in global gene expression levels as well as the need for the identification of new molecular markers associated with the development and progression of liver disease. Furthermore, if intervention is expected to be successful in halting or slowing down liver disease, means of accurately assessing the early manifestations of liver disease need to be established. One way to accurately assess the early manifestations of liver disease is to identify markers which are uniquely associated with disease progression (see for example Kim *et al.* (2001) *Oncogene* 20: 4568-4575). Likewise, the development of therapeutics to prevent or stop the progression of liver disease relies on the identification of genes responsible for the cancerous transformation of liver cells and the growth of cancerous liver cells.

To date, researchers have been able to identify a few genetic alterations believed to underlie tumor development. These genetic alterations include amplification of oncogenes and mutations that result in the loss of tumor suppressor genes. Tumor suppressor genes are genes that, in their wild-type alleles, express proteins that suppress abnormal cellular proliferation. When the gene coding for a tumor suppressor protein is mutated or deleted, the resulting mutant protein or the complete lack of tumor suppressor protein expression may fail to correctly regulate cellular proliferation, and abnormal proliferation may take place, particularly if there is already existing damage to the cellular regulatory mechanism. A number of well-studied human tumors and tumor cell lines have missing or non-functional tumor suppressor genes. Examples of tumor suppressor genes include, but are not limited to, the retinoblastoma susceptibility gene or RB gene, the p53 gene, the deletion in colon carcinoma (DCC) gene and the neurofibromatosis type 1 (NF-1) tumor suppressor gene (Weinberg, R. A. *Science*, 1991, 254:1138-1146). Loss of function or inactivation of tumor suppressor genes may play a central role in the initiation and/or progression of a significant number of human cancers.

Classification of heterogeneous populations of tumor types is a daunting task; yet, initial studies utilizing gene expression patterns to identify subtypes of cancer produced rather intriguing results (see Perou *et al.*, *Proc Natl Acad Sci U S A* 96:9212-9217, 1999; Golub *et al.*, *Science* 286:531-537, 1999; Alizadeh *et al.*, *Nature* 403:503-511, 2000; Alon *et al.* *Proc Natl Acad Sci U S A* 96:6745-6750, 1999; and Bittner *et al.*, *Nature* 406:536-540, 2000). Molecular classification of B-cell lymphoma by gene

expression profiling elucidated clinically distinct diffuse large-B-cell lymphoma subgroups (see Alizadeh *supra*). Stratification of patients based on their distinctive gene expression profiles may allow researchers to precisely group similar patient populations for evaluating chemotherapeutic agents. The more homogenous population of patients decreases the variability of patient-to-patient responses leading to the development of agents capable of eradicating specific subtypes of cancers previously unknown using standard classification techniques.

The utilization of gene expression profiles to classify tumors, to identify drug targets, to identify diagnostic markers and/or to gain further insights into the consequences of chemotherapeutic treatments could facilitate the design of more efficacious patient-specific stratagems for treating a variety of cancers. In breast cancer, studies utilizing limited numbers of genes (8,102 genes) have classified tumors into subtypes based on gene expression profiles, and this study indicated a diversity of molecular phenotypes associated with breast tumors (Perou *et al.*, *Nature* 406:747-752, 2000). The advent of cDNA and oligonucleotide arrays has enabled researchers to map tissue-specific expression levels for thousands of genes (Alon *et al.*, *Proc Natl Acad Sci USA* 96:6745-6750, 1999; Iyer *et al.*, *Science* 283:83-87, 1999; Khan *et al.*, *Cancer Res* 58:5009-5013, 1998; Lee *et al.*, *Science* 285:1390-1393, 1999; Wang *et al.*, *Gene* 229:101-108, 1999; Whitney *et al.*, *Ann Neurol* 46:425-428, 1999). The study by Martin *et al.* (*Cancer Res* 60:2232-2238, 2000) used a custom microarray composed of 124 genes discovered by differential display associated with either normal breast epithelial cells or from the MDA-MB-435 malignant breast tumor cell line. Using the custom microarray, researchers examined the relationship between expression patterns discovered by clustering a number of genes with clinical stages of breast cancer indicating that gene expression patterns were capable of grouping breast tumors into distinct categories (Martin *et al.*, *supra*).

Although these studies have demonstrated that expression profiling may be used to produce improvements in diagnosis of human diseases such as cancer, as well as in the development of improved therapeutic strategies, further studies are needed. Accordingly, there remains a need in the art for materials and methods that permit a more accurate diagnosis of hepatic carcinomas, as well as of other chronic liver diseases. In addition, there remains a need in the art for methods to treat and methods to identify agents that can effectively treat liver disease. The present invention meets these and other needs.

## SUMMARY OF THE INVENTION

The present invention is based on the discovery of the genes and their expression profiles associated with various types and stages of liver disease, in particular hepatocellular carcinoma (HCC), chronic hepatitis (CH) and liver cirrhosis (LC).

5       The invention includes methods of diagnosing liver disease in a patient comprising the step of detecting the level of expression in a tissue sample of one or more genes from Table 1; wherein differential expression of the genes in Table 1 is indicative of liver disease. The invention also includes methods of detecting the progression of liver disease. For instance, methods of the invention include detecting the progression of liver  
10   disease in a patient comprising the step of detecting the level of expression in a tissue sample of one or more genes from Table 1; wherein differential expression of the genes in Table 1 is indicative of liver disease progression. In some preferred embodiments, PCA analysis based on all or a portion of the group of genes identified in Table 1 may be used to differentiate between the different stages of liver disease, such as in the metastasis of  
15   liver carcinomas. In some preferred embodiments, one or more genes may be selected from Table 1.

In some aspects, the present invention provides a method of monitoring the treatment of a patient with liver disease, comprising administering a pharmaceutical composition to the patient, preparing a gene expression profile from a cell or tissue sample  
20   from the patient and comparing the patient gene expression profile to a gene expression profile from a cell population comprising normal liver cells, or to a gene expression profile from a cell population comprising disease state liver cells, or to both. In some preferred embodiments, the gene profile will include the expression level of one or more genes in Table 1.

25       Another aspect of the present invention includes a method of treating a patient with liver disease, comprising administering to the patient a pharmaceutical composition, wherein the composition alters the expression of at least one gene in Table 1, preparing a gene expression profile from a cell or tissue sample from the patient comprising diseased cells and comparing the patient expression profile to a gene expression profile from an  
30   untreated cell population comprising disease state liver cells.

In another aspect, the present invention provides a method of detecting the progression of carcinogenesis in a patient, comprising detecting the level of expression in

a tissue sample of one or more genes from Table 1; wherein differential expression of the genes in Table 1 is indicative of hepatic carcinogenesis.

The invention further includes methods of screening for an agent capable of modulating the onset or progression of liver disease, comprising the steps of exposing a cell to the agent; and detecting the expression level of one or more genes from Table 1. In some embodiments, the liver disease may be a hepatocellular carcinoma. In some preferred embodiments, one or more genes may be selected from a group consisting of those listed in Table 1. In some preferred methods, it may be desirable to detect all or nearly all of the genes in the tables.

The invention further includes compositions comprising at least two oligonucleotides, wherein each of the oligonucleotides comprises a sequence that specifically hybridizes to a gene in Table 1, as well as solid supports comprising at least two probes, wherein each of the probes comprises a sequence that specifically hybridizes to a gene in Table 1. In some preferred embodiments, one or more genes may be selected from a group consisting of those listed in Table 1.

The invention further includes computer systems comprising a database containing information identifying the expression level in liver tissue of a set of genes comprising at least two genes in Table 1 and a user interface to view the information. In some preferred embodiments, one or more genes may be selected from a group consisting of those listed in Table 1. The database may further include sequence information for the genes, information identifying the expression level for the set of genes in non-cancerous liver tissue and in cancerous liver tissue and may contain links to external databases such as GenBank.

Lastly, the invention includes methods of using the databases, such as methods of using the disclosed computer systems to present information identifying the expression level in a tissue or cell of at least one gene in Table 1, comprising the step of comparing the expression level of at least one gene in Table 1 in the tissue or cell to the level of expression of the gene in the database. In some preferred embodiments, one or more genes may be selected from a group consisting of those listed in Table 1.

### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Many biological functions are accomplished by altering the expression of various genes through transcriptional (e.g., through control of initiation, provision of RNA

precursors, RNA processing, etc.) and/or translational control. For example, fundamental biological processes such as cell cycle, cell differentiation and cell death, are often characterized by the variations in the expression levels of groups of genes.

Changes in gene expression also are associated with pathogenesis. For example,  
5 the lack of sufficient expression of functional tumor suppressor genes and/or the over expression of oncogene/protooncogenes could lead to tumorigenesis or hyperplastic growth of cells (Marshall, *Cell* 64:313-326, 1991; Weinberg, *Science*, 254:1138-1146, 1991). Thus, changes in the expression levels of particular genes (*e.g.*, oncogenes or tumor suppressors) serve as signposts for the presence and progression of various diseases.

10 Monitoring changes in gene expression may also provide certain advantages during drug screening and development. Often drugs are pre-screened for the ability to interact with a major target without regard to other effects the drugs have on cells. Often such other effects cause toxicity in the whole animal, which prevent the development and use of the potential drug.

15 Using pairs of samples from subjects, applicants have examined samples from diseased but non-cancerous liver tissue and from cancerous liver tissue to identify global changes in gene expression between tumor biopsies and surrounding non-cancerous tissue. Diseased but non-cancerous liver tissue was either inflamed tissue from chronic viral hepatitis patients or fibrotic tissue from liver cirrhosis patients. Non-cancerous tissue was  
20 removed from a point in the liver adjacent to a tumor biopsy site. These global changes in gene expression, also referred to as expression profiles, provide useful markers for diagnostic uses as well as markers that can be used to monitor disease states, disease progression, drug toxicity, drug efficacy and drug metabolism.

The gene expression profiles described herein were derived from diseased liver  
25 biopsy samples from Korean patients 34-65 years old. These patients had been diagnosed with chronic hepatitis or cirrhosis and, in each case, had subsequently developed liver cancer. The disease state associated with each sample is indicated in Table 2.

The present invention provides compositions and methods to detect the level of expression of genes that may be differentially expressed dependent upon the state of the  
30 cell, *i.e.*, non-cancerous versus cancerous. These expression profiles of genes provide molecular tools for evaluating toxicity, drug efficacy, drug metabolism, development, and disease monitoring. Changes in the expression profile from a baseline profile can be used as an indication of such effects. Those skilled in the art can use any of a variety of known

techniques to evaluate the expression of one or more of the genes and/or gene fragments identified in the instant application in order to observe changes in the expression profile in a tissue or sample of interest.

### *Definitions*

5           In the description that follows, numerous terms and phrases known to those skilled in the art are used. In the interest of clarity and consistency of interpretation, the definitions of certain terms and phrases are provided.

          As used herein, the phrase "detecting the level of expression" includes methods that quantify expression levels as well as methods that determine whether a gene of  
10       interest is expressed at all. Thus, an assay which provides a yes or no result without necessarily providing quantification of an amount of expression is an assay that requires "detecting the level of expression" as that phrase is used herein.

          As used herein, oligonucleotide sequences that are complementary to one or more of the genes described herein, refers to oligonucleotides that are capable of hybridizing  
15       under stringent conditions to at least part of the nucleotide sequence of said genes. Such hybridizable oligonucleotides will typically exhibit at least about 75% sequence identity at the nucleotide level to said genes, preferably about 80% or 85% sequence identity or more preferably about 90% or 95% or more nucleotide sequence identity to said genes.

          "Bind(s) substantially" refers to complementary hybridization between a probe  
20       nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target polynucleotide sequence.

          The terms "background" or "background signal intensity" refer to hybridization signals resulting from non-specific binding, or other interactions, between the labeled  
25       target nucleic acids and components of the oligonucleotide array (e.g., the oligonucleotide probes, control probes, the array substrate, etc.). Background signals may also be produced by intrinsic fluorescence of the array components themselves. A single background signal can be calculated for the entire array, or a different background signal may be calculated for each target nucleic acid. In a preferred embodiment, background is  
30       calculated as the average hybridization signal intensity for the lowest 5% to 10% of the probes in the array, or, where a different background signal is calculated for each target gene, for the lowest 5% to 10% of the probes for each gene. Of course, one of skill in the

art will appreciate that where the probes to a particular gene hybridize well and thus appear to be specifically binding to a target sequence, they should not be used in a background signal calculation. Alternatively, background may be calculated as the average hybridization signal intensity produced by hybridization to probes that are not complementary to any sequence found in the sample (e.g., probes directed to nucleic acids of the opposite sense or to genes not found in the sample such as bacterial genes where the sample is mammalian nucleic acids). Background can also be calculated as the average signal intensity produced by regions of the array that lack any probes at all.

The phrase "hybridizing specifically to" refers to the binding, duplexing or hybridizing of a molecule substantially to or only to a particular nucleotide sequence or sequences under stringent conditions when that sequence is present in a complex mixture (e.g., total cellular) DNA or RNA.

Assays and methods of the invention may utilize available formats to simultaneously screen at least about 100, preferably about 1000, more preferably about 10,000 and most preferably about 1,000,000 or more different nucleic acid hybridizations.

The terms "mismatch control" or "mismatch probe" refer to a probe whose sequence is deliberately selected not to be perfectly complementary to a particular target sequence. For each mismatch (MM) control in a high-density array there typically exists a corresponding perfect match (PM) probe that is perfectly complementary to the same particular target sequence. The mismatch may comprise one or more bases that are not complementary to the corresponding bases of the target sequence.

While the mismatch(s) may be located anywhere in the mismatch probe, terminal mismatches are less desirable as a terminal mismatch is less likely to prevent hybridization of the target sequence. In a particularly preferred embodiment, the mismatch is located at or near the center of the probe such that the mismatch is most likely to destabilize the duplex with the target sequence under the test hybridization conditions.

The term "perfect match probe" refers to a probe that has a sequence that is perfectly complementary to a particular target sequence. The test probe is typically perfectly complementary to a portion (subsequence) of the target sequence. The perfect match (PM) probe can be a "test probe", a "normalization control" probe, an expression level control probe and the like. A perfect match control or perfect match probe is, however, distinguished from a "mismatch control" or "mismatch probe."



As used herein a "probe" is defined as a nucleic acid, preferably an oligonucleotide, capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein, a probe may include natural (i.e., A, G, U, C or T) or modified bases (7-deazaguanosine, inosine, *etc.*). In addition, the bases in probes may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridization. Thus, probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages.

The term "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, but with only insubstantial hybridization to other sequences or to other sequences such that the difference may be identified. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH.

Typically, stringent conditions will be those in which the salt concentration is at least about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotide). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

The "percentage of sequence identity" or "sequence identity" is determined by comparing two optimally aligned sequences or subsequences over a comparison window or span, wherein the portion of the polynucleotide sequence in the comparison window may optionally comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical subunit (e.g., nucleic acid base or amino acid residue) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Percentage sequence

identity when calculated using the programs GAP or BESTFIT (see below) is calculated using default gap weights.

Homology or identity may be determined by BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al.*, *Proc Natl Acad Sci USA* 87:2264-2268, 1990 and Altschul, *J Mol Evol* 36:290-300, 1993, fully incorporated by reference) which are tailored for sequence similarity searching. The approach used by the BLAST program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al.*, (*Nature Genet* 6:119-129, 1994) which is fully incorporated by reference. The search parameters for histogram, descriptions, alignments, expect (i.e., the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, *Proc Natl Acad Sci USA* 89:10915-10919, 1992, fully incorporated by reference). Four blastn parameters were adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink<sup>th</sup> position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings were Q=9; R=2; wink=1; and gapw=32. A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

## 25 *Uses of Differentially Expressed Genes*

The present invention identifies those genes differentially expressed between cancerous and non-cancerous liver tissue. One of skill in the art can select one or more of the genes identified as being differentially expressed in Table 1 and use the information and methods provided herein to interrogate or test a particular sample. For a particular interrogation of two conditions or sources, it may be desirable to select those genes which display a great deal of difference in the expression pattern between the two conditions or sources. In other instances, it may be appropriate to select genes whose expression

changes only slightly between the two conditions. At least a 1.5-fold difference may be desirable, but a three-fold, five-fold or ten-fold difference may be preferred in some instances. The data are subjected to statistical evaluation to ensure that the observed differences and the disease association are statistically significant. Interrogations of the genes or proteins can be performed to yield different information.

#### *Diagnostic Uses for the Liver Cancer Markers*

As described herein, the genes and gene expression information provided in Table 1 may be used as diagnostic markers for the prediction or identification of a disease state of liver tissue. For instance, a liver tissue sample or other sample from a patient may be assayed by any of the methods known to those skilled in the art, and the expression levels from one or more genes from Table 1 may be compared to the expression levels found in non-cancerous liver tissue, cancerous liver tissue or both. Expression profiles generated from the tissue or other samples that substantially resemble an expression profile from non-cancerous or cancerous liver tissue may be used, for instance, to aid in disease diagnosis. Comparison of the expression data, as well as available sequence or other information, may be done by a researcher or diagnostician or may be done with the aid of a computer and databases as described herein.

#### *Use of the Liver Cancer Markers for Monitoring Disease Progression*

Molecular expression markers for liver disease can be used to confirm the type and progression of disease made on the basis of morphological criteria. For example, non-cancerous liver tissue could be distinguished from cancerous tissue based on the level and type of genes expressed in a tissue sample. In some situations, identifications of cell type or source is ambiguous based on classical criteria. In these situations, the molecular expression markers of the present invention are useful for identifying the region of the liver from which a sample came, as well as whether or not normal levels of gene expression have been altered (signs of metabolic disturbances).

In addition, progression of hepatic carcinoma to new areas of the liver can be monitored by following the expression patterns of the involved genes using the molecular expression markers of the present invention. Monitoring of the efficacy of certain drug regimens can also be accomplished by following the expression patterns of the molecular expression markers.

As described above, the genes and gene expression information provided in Table 1 may also be used as markers for the direct monitoring of disease progression, for instance, the development of liver cancer. A liver tissue sample or other sample from a patient may be assayed by any of the methods known to those of skill in the art, and the expression levels in the sample from a gene or genes from Table 1 may be compared to the expression levels found in non-cancerous liver tissue, tissue from a hepatic carcinoma or both. Comparison of the expression data, as well as available sequence or other information may be done by a researcher or diagnostician or may be done with the aid of a computer and databases as described herein.

#### *Use of the Liver Cancer Markers for Drug Screening*

According to the present invention, potential drugs can be screened to determine if application of the drug alters the expression of one or more of the genes identified herein. This may be useful, for example, in determining whether a particular drug is effective in treating a particular patient with liver disease. In the case where a gene's expression is affected by the potential drug such that its level of expression returns to normal, the drug is indicated in the treatment of liver cancer. Similarly, a drug which causes expression of a gene which is not normally expressed by healthy liver cells may be contra-indicated in the treatment of liver cancer.

According to the present invention, the genes identified in Table 1 may also be used as markers to evaluate the effects of a candidate drug or agent on a cell, particularly a cell undergoing malignant transformation, for instance, a liver cancer cell or tissue sample. A candidate drug or agent can be screened for the ability to stimulate the transcription or expression of a given marker or markers (drug targets) or to down-regulate or inhibit the transcription or expression of a marker or markers. According to the present invention, one can also compare the specificity of a drug's effects by looking at the number of markers affected by the drug and comparing them to the number of markers affected by a different drug. A more specific drug will affect fewer transcriptional targets. Similar sets of markers identified for two drugs indicates a similarity of effects.

Assays to monitor the expression of a marker or markers as defined in Table 1 may utilize any available means of monitoring for changes in the expression level of the nucleic acids of the invention. As used herein, an agent is said to modulate the expression

of a nucleic acid of the invention if it is capable of up- or down-regulating expression of the nucleic acid in a cell.

Agents that are assayed in the above methods can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of a protein of the invention alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis which takes into account the sequence of the target site and/or its conformation in connection with the agents action. Agents can be selected or designed by utilizing the peptide sequences that make up these sites. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to or a derivative of any functional consensus site.

The agents of the present invention can be, as examples, peptides, small chemical molecules, vitamin derivatives, as well as carbohydrates, lipids, oligonucleotides and covalent and non-covalent combinations thereof. Dominant negative proteins, DNA encoding these proteins, antibodies to these proteins, peptide fragments of these proteins or mimics of these proteins may be introduced into cells to affect function. "Mimic" as used herein refers to the modification of a region or several regions of a peptide molecule to provide a structure chemically different from the parent peptide but topographically and functionally similar to the parent peptide (see Grant, in Molecular Biology and Biotechnology, Meyers (ed.), VCH Publishers, 1995). A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

#### *Use of the Liver Cancer Markers as Therapeutic Agents*

Agents that up- or down-regulate or modulate the expression of the nucleic acid molecules of Table 1, or at least one activity of a protein encoded by the nucleic acid molecules of Table 1, such as agonists or antagonists, may be used to modulate biological and pathologic processes associated with the function and activity of the proteins encoded by these nucleic acid molecules. The agents can be the nucleic acid molecules of Table 1

themselves, the encoded proteins, or portions of these molecules, such as all or part of the open reading frames of these nucleic acid molecules.

Anti-sense oligonucleotide molecules derived from the nucleic acid sequences of Table 1 may also be used to down-regulate the expression of one or more of the genes in Table 1 that are expressed at elevated levels in liver cancer, the use of antisense gene therapy being an example. Down-regulation of expression of one or more of the genes of Table 1 is accomplished by administering an effective amount of antisense oligonucleotides. These antisense molecules can be fashioned from the DNA sequences of these genes or sequences containing various mutations, deletions, insertions or spliced variants. Isolated RNA or DNA sequences derived from these genes may also be used therapeutically in gene therapy. These agents may be used to induce gene expression in liver cancers associated with an absence of or considerably decreased expression of one or more of the proteins encoded by genes in Table 1.

As used herein, a subject can be any mammal, so long as the mammal is in need of modulation of a pathological or biological process mediated by a gene of the invention. The term "mammal" is defined as an individual belonging to the class Mammalia. The invention is particularly useful in the treatment of human subjects.

Pathological processes refer to a category of biological processes which produce a deleterious effect. For example, expression of a gene of the invention may be associated with hyperplasia in the liver, in particular malignant hyperplasia. As used herein, an agent is said to modulate a pathological process when the agent reduces the degree or severity of the process. For instance, liver cancer may be prevented or disease progression modulated by the administration of agents which up- or down-regulate or modulate in some way the expression or at least one activity of a gene of the invention.

The agents of the present invention can be provided alone, or in combination with other agents that modulate a particular pathological process. For example, an agent of the present invention can be administered in combination with other known drugs. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act at the same time.

The agents of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage

administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The present invention further provides compositions containing one or more agents which modulate expression or at least one activity of a protein of the invention. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise 0.1 to 100  $\mu\text{g/kg}$  body wt. The preferred dosages comprise 0.1 to 10  $\mu\text{g/kg}$  body wt. The most preferred dosages comprise 0.1 to 1  $\mu\text{g/kg}$  body wt.

In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, *e.g.*, sesame oil, or synthetic fatty acid esters, *e.g.*, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be coadministered along with other compounds typically prescribed for these conditions

according to generally accepted medical practice. The compounds of this invention can be utilized in vivo, ordinarily in mammals, such as humans, rats, mice, dogs, cats, sheep, horses, cattle and pigs, or in vitro.

#### *Assay Formats*

5           The genes identified as being differentially expressed in liver disease may be used in a variety of nucleic acid detection assays to detect or quantify the expression level of a gene or multiple genes in a given sample. For example, traditional Northern blotting, nuclease protection, RT-PCR and differential display methods may be used for detecting gene expression levels. In methods where small numbers of genes are assayed, such as 5-  
10 50 genes, high-throughput PCR may be used.

          The protein products of the genes identified herein can also be assayed to determine the amount of expression. Methods for assaying for a protein include Western blot, immunoprecipitation and radioimmunoassay. In some methods, it is preferable to assay the mRNA as an indication of expression. Methods for assaying for mRNA include  
15 Northern blots, slot blots, dot blots, and hybridization to an ordered array of oligonucleotides. Any method for specifically and quantitatively measuring a specific protein or mRNA or DNA product can be used. However, methods and assays of the invention are most efficiently designed with array or chip hybridization-based methods for detecting the expression of a large number of genes.

20           Any hybridization assay format may be used, including solution-based and solid support-based assay formats. A preferred solid support is a high density array also known as a DNA chip or a gene chip. One variation of the DNA chip contains hundreds of thousands of discrete microscopic channels that pass completely through it. Probe molecules are attached to the inner surface of these channels, and molecules from the  
25 samples to be tested flow throughout the channels, coming into close proximity with the probes for hybridization. In one assay format, gene chips containing probes to at least two genes from Table 1 may be used to directly monitor or detect changes in gene expression in the treated or exposed cell as described herein.

          The genes of the present invention may be assayed in any convenient sample  
30 form. For example, samples may be assayed in the form mRNA or reverse transcribed mRNA. Samples may be cloned or not, and the samples or individual genes may be amplified or not. The cloning itself does not appear to bias the representation of genes



within a population. However, it may be preferable to use polyA<sup>+</sup> RNA as a source, as it can be used with less processing steps. In some embodiments, it may be preferable to assay the protein or peptide expressed by the gene.

The sequences of the expression marker genes of Table 1 are available in the public databases. Table 1 provides the Accession number, Sequence Number ID and name for each of the sequences. The sequences of the genes in GenBank are herein expressly incorporated by reference in their entirety (see [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

Additional assay formats may be used to monitor the ability of the agent to modulate the expression of a gene identified in Table 1. For instance, as described above, mRNA expression may be monitored directly by hybridization of probes to the nucleic acids of the invention. Cell lines are exposed to an agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook *et al.*, Molecular Cloning - A Laboratory Manual, Third ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001. In some embodiments, it may be desirable to amplify one or more of the RNA molecules isolated prior to application of the RNA to the gene chip. Using techniques well known in the art, the RNA may be reverse transcribed and amplified in the form of DNA or may be reverse transcribed into DNA and the DNA used as a template for transcription to generate recombinant RNA. Any method that results in the production of a sufficient quantity of nucleic acid to be hybridized effectively to the gene chip may be used.

In another format, cell lines that contain reporter gene fusions between the open reading frame and/or the 3' or 5' regulatory regions of a gene in Table 1 and any assayable fusion partner may be prepared. Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.*, *Anal Biochem* 188:245-254, 1990). Cell lines containing the reporter gene fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of the nucleic acid.

In another assay format, cells or cell lines are first identified which express one or more of the gene products of the invention physiologically. Cells and/or cell lines so identified would preferably comprise the necessary cellular machinery to ensure that the transcriptional and/or translational apparatus of the cells would faithfully mimic the

response of normal or cancerous liver tissue to an exogenous agent. Such machinery would likely include appropriate surface transduction mechanisms and/or cytosolic factors. Such cell lines may be, but are not required to be, derived from liver tissue. The cells and/or cell lines may then be contacted with an agent and the expression of one or more of the genes of interest may then be assayed. The genes may be assayed at the mRNA level and/or at the protein level.

In some embodiments, such cells or cell lines may be transduced or transfected with an expression vehicle (e.g., a plasmid or viral vector) containing an expression construct comprising an operable 5'-promoter containing end of a gene of interest identified in Table 1 fused to one or more nucleic acid sequences encoding one or more antigenic fragments. The construct may comprise all or a portion of the coding sequence of the gene of interest which may be positioned 5'- or 3'- to a sequence encoding an antigenic fragment. The coding sequence of the gene of interest may be translated or untranslated after transcription of the gene fusion. At least one antigenic fragment may be translated. The antigenic fragments are selected so that the fragments are under the transcriptional control of the promoter of the gene of interest and are expressed in a fashion substantially similar to the expression pattern of the gene of interest. The antigenic fragments may be expressed as polypeptides whose molecular weight can be distinguished from the naturally occurring polypeptides.

In some embodiments, gene products of the invention may further comprise an immunologically distinct tag. Such a process is well known in the art (see Sambrook *et al.*, *supra*). Cells or cell lines transduced or transfected as outlined above are then contacted with agents under appropriate conditions; for example, the agent comprises a pharmaceutically acceptable excipient and is contacted with cells comprised in an aqueous physiological buffer such as phosphate buffered saline (PBS) at physiological pH, Eagles balanced salt solution (BSS) at physiological pH, PBS or BSS comprising serum or conditioned media comprising PBS or BSS and serum incubated at 37°C. Said conditions may be modulated as deemed necessary by one of skill in the art. Subsequent to contacting the cells with the agent, said cells will be disrupted and the polypeptides of the lysate are fractionated such that a polypeptide fraction is pooled and contacted with an antibody to be further processed by immunological assay (e.g., ELISA, immunoprecipitation or Western blot). The pool of proteins isolated from the "agent-

contacted" sample will be compared with a control sample where only the excipient is contacted with the cells and an increase or decrease in the immunologically generated signal from the "agent-contacted" sample compared to the control will be used to distinguish the effectiveness of the agent.

5 Another embodiment of the present invention provides methods for identifying agents that modulate the levels, concentration or at least one activity of a protein(s) encoded by the genes in Table 1. Such methods or assays may utilize any means of monitoring or detecting the desired activity.

10 In one format, the relative amounts of a protein of the invention produced in a cell population that has been exposed to the agent to be tested may be compared to the amount produced in an un-exposed control cell population. In this format, probes such as specific antibodies are used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates may be prepared from the exposed  
15 cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe, such as a specific antibody.

#### *Probe design*

Probes based on the sequences of the genes described herein may be prepared by any commonly available method. Oligonucleotide probes for assaying the tissue or cell  
20 sample are preferably of sufficient length to specifically hybridize only to appropriate, complementary genes or transcripts. Typically the oligonucleotide probes will be at least 10, 12, 14, 16, 18, 20 or 25 nucleotides in length. In some cases longer probes of at least 30, 40, or 50 nucleotides will be desirable.

One of skill in the art will appreciate that an enormous number of array designs  
25 are suitable for the practice of this invention. The high density array will typically include a number of probes that specifically hybridize to the sequences of interest. See WO 99/32660 for methods of producing probes for a given gene or genes. In addition, in a preferred embodiment, the array will include one or more control probes.

High density array chips of the invention include "test probes." Test probes may  
30 be oligonucleotides that range from about 5 to about 500 or about 5 to about 50 nucleotides, more preferably from about 10 to about 40 nucleotides and most preferably from about 15 to about 40 nucleotides in length. In other particularly preferred

embodiments, the probes are about 20 or 25 nucleotides in length. In another preferred embodiment, test probes are double or single strand DNA sequences. DNA sequences may be isolated or cloned from natural sources or amplified from natural sources using natural nucleic acid as templates. These probes have sequences complementary to particular subsequences of the genes whose expression they are designed to detect. Thus, the test probes are capable of specifically hybridizing to the target nucleic acid they are to detect.

In addition to test probes that bind the target nucleic acid(s) of interest, the high density array can contain a number of control probes. The control probes fall into three categories referred to herein as (1) normalization controls; (2) expression level controls; and (3) mismatch controls.

Normalization controls are oligonucleotide or other nucleic acid probes that are complementary to labeled reference oligonucleotides or other nucleic acid sequences that are added to the nucleic acid sample. The signals obtained from the normalization controls after hybridization provide a control for variations in hybridization conditions, label intensity, "reading" efficiency and other factors that may cause the signal of a perfect hybridization to vary between arrays. In a preferred embodiment, signals (e.g., fluorescence intensity) read from all other probes in the array are divided by the signal (e.g., fluorescence intensity) from the control probes thereby normalizing the measurements.

Virtually any probe may serve as a normalization control. However, it is recognized that hybridization efficiency varies with base composition and probe length. Preferred normalization probes are selected to reflect the average length of the other probes present in the array, however, they can be selected to cover a range of lengths. The normalization control(s) can also be selected to reflect the (average) base composition of the other probes in the array, however in a preferred embodiment, only one or a few probes are used and they are selected such that they hybridize well (i.e., no secondary structure) and do not match any target-specific probes.

Expression level controls are probes that hybridize specifically with constitutively expressed genes in the biological sample. Virtually any constitutively expressed gene provides a suitable target for expression level controls. Typical expression level control probes have sequences complementary to subsequences of constitutively

expressed "housekeeping genes" including, but not limited to the  $\beta$ -actin gene, the transferrin receptor gene, the GAPDH gene, and the like.

Mismatch controls may also be provided for the probes to the target genes, for expression level controls or for normalization controls. Mismatch controls are oligonucleotide probes or other nucleic acid probes identical to their corresponding test or control probes except for the presence of one or more mismatched bases. A mismatched base is a base selected so that it is not complementary to the corresponding base in the target sequence to which the probe would otherwise specifically hybridize. One or more mismatches are selected such that under appropriate hybridization conditions (e.g., stringent conditions) the test or control probe would be expected to hybridize with its target sequence, but the mismatch probe would not hybridize (or would hybridize to a significantly lesser extent). Preferred mismatch probes contain a central mismatch. Thus, for example, where a probe is a twenty-mer, a corresponding mismatch probe may have the identical sequence except for a single base mismatch (e.g., substituting a G, a C or a T for an A) at any of positions 6 through 14 (the central mismatch).

Mismatch probes thus provide a control for non-specific binding or cross hybridization to a nucleic acid in the sample other than the target to which the probe is directed. Mismatch probes also indicate whether a hybridization is specific or not. For example, if the target is present the perfect match probes should be consistently brighter than the mismatch probes. In addition, if all central mismatches are present, the mismatch probes can be used to detect a mutation. The difference in intensity between the perfect match and the mismatch probe ( $I(\text{PM}) - I(\text{MM})$ ) provides a good measure of the concentration of the hybridized material.

#### *Nucleic Acid Samples*

As is apparent to one of ordinary skill in the art, nucleic acid samples used in the methods and assays of the invention may be prepared by any available method or process. Methods of isolating total mRNA are also well known to those of skill in the art. For example, methods of isolation and purification of nucleic acids are described in detail in Chapter 3 of Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 24, Hybridization With Nucleic Acid Probes: Theory and Nucleic Acid Probes, P. Tijssen (ed.) Elsevier Press, New York, 1993. Such samples include RNA samples, but also include cDNA synthesized from a mRNA sample isolated from a cell or tissue of interest.

Such samples also include DNA amplified from the cDNA, and an RNA transcribed from the amplified DNA. One of skill in the art would appreciate that it may be desirable to inhibit or destroy RNase present in homogenates before homogenates can be used.

Biological samples may be of any biological tissue or fluid or cells from any  
5 organism as well as cells raised *in vitro*, such as cell lines and tissue culture cells.  
Frequently the sample will be a "clinical sample" which is a sample derived from a patient.  
Typical clinical samples include, but are not limited to, liver tissue biopsy, sputum, blood,  
blood-cells (e.g., white cells), tissue or fine needle biopsy samples, urine, peritoneal fluid,  
and pleural fluid, or cells therefrom. Biological samples may also include sections of  
10 tissues, such as frozen sections or formalin fixed sections taken for histological purposes.

#### *Solid Supports*

Solid supports containing oligonucleotide probes for differentially expressed genes can be any solid or semisolid support material known to those skilled in the art. Suitable examples include, but are not limited to, membranes, filters, tissue culture dishes,  
15 polyvinyl chloride dishes, beads, test strips, silicon or glass based chips and the like.  
Suitable glass wafers and hybridization methods are widely available, for example, those disclosed by Beattie (WO 95/11755). Any solid surface to which oligonucleotides can be bound, either directly or indirectly, either covalently or non-covalently, can be used. In some embodiments, it may be desirable to attach some oligonucleotides covalently and  
20 others non-covalently to the same solid support.

A preferred solid support is a high density array or DNA chip. These contain a particular oligonucleotide probe in a predetermined location on the array. Each predetermined location may contain more than one molecule of the probe, but each molecule within the predetermined location has an identical sequence. Such  
25 predetermined locations are termed features. There may be, for example, from 2, 10, 100, 1000 to 10,000, 100,000 or 400,000 of such features on a single solid support. The solid support, or the area within which the probes are attached may be on the order of a square centimeter.

Oligonucleotide probe arrays for expression monitoring can be made and used  
30 according to any techniques known in the art (see for example, Lockhart *et al.*, *Nat Biotechnol* 14:1675-1680, 1996; McGall *et al.*, *Proc Nat Acad Sci USA* 93: 13555-13460, 1996). Such probe arrays may contain at least two or more oligonucleotides that are

complementary to or hybridize to two or more of the genes described herein. Such arrays may also contain oligonucleotides that are complementary or hybridize to at least 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 50, 70 or more the genes described herein.

Methods of forming high density arrays of oligonucleotides with a minimal number of synthetic steps are known. The oligonucleotide analogue array can be synthesized on a solid substrate by a variety of methods, including, but not limited to, light-directed chemical coupling, and mechanically directed coupling (see Pirrung *et al.*, (1992) U.S. Patent No. 5,143, 854; Fodor *et al.*, (1998) U.S. Patent No. 5,800,992; Chee *et al.*, (1998) 5,837,832).

In brief, the light-directed combinatorial synthesis of oligonucleotide arrays on a glass surface proceeds using automated phosphoramidite chemistry and chip masking techniques. In one specific implementation, a glass surface is derivatized with a silane reagent containing a functional group, e.g., a hydroxyl or amine group blocked by a photolabile protecting group. Photolysis through a photolithographic mask is used selectively to expose functional groups which are then ready to react with incoming 5' photoprotected nucleoside phosphoramidites. The phosphoramidites react only with those sites which are illuminated (and thus exposed by removal of the photolabile blocking group). Thus, the phosphoramidites only add to those areas selectively exposed from the preceding step. These steps are repeated until the desired array of sequences have been synthesized on the solid surface. Combinatorial synthesis of different oligonucleotide analogues at different locations on the array is determined by the pattern of illumination during synthesis and the order of addition of coupling reagents.

In addition to the foregoing, additional methods which can be used to generate an array of oligonucleotides on a single substrate are described in Fodor *et al.* WO 93/09668.

High density nucleic acid arrays can also be fabricated by depositing pre-made or natural nucleic acids in predetermined positions. Synthesized or natural nucleic acids are deposited on specific locations of a substrate by light directed targeting and oligonucleotide directed targeting. Another embodiment uses a dispenser that moves from region to region to deposit nucleic acids in specific spots.

### Hybridization

Nucleic acid hybridization simply involves contacting a probe and target nucleic acid under conditions where the probe and its complementary target can form stable

hybrid duplexes through complementary base pairing (see Lockhart *et al.*, (1999) WO 99/32660). The nucleic acids that do not form hybrid duplexes are then washed away leaving the hybridized nucleic acids to be detected, typically through detection of an attached detectable label. It is generally recognized that nucleic acids are denatured by increasing the temperature or decreasing the salt concentration of the buffer containing the nucleic acids. Under low stringency conditions (*e.g.*, low temperature and/or high salt) hybrid duplexes (*e.g.*, DNA-DNA, RNA-RNA or RNA-DNA) will form even where the annealed sequences are not perfectly complementary. Thus, specificity of hybridization is reduced at lower stringency. Conversely, at higher stringency (*e.g.*, higher temperature or lower salt) successful hybridization requires fewer mismatches. One of skill in the art will appreciate that hybridization conditions may be selected to provide any degree of stringency. In a preferred embodiment, hybridization is performed at low stringency, in this case in 6× SSPE-T at 37°C (0.005% Triton x-100) to ensure hybridization and then subsequent washes are performed at higher stringency (*e.g.*, 1× SSPE-T at 37°C) to eliminate mismatched hybrid duplexes. Successive washes may be performed at increasingly higher stringency (*e.g.*, down to as low as 0.25× SSPET at 37°C to 50°C) until a desired level of hybridization specificity is obtained. Stringency can also be increased by addition of agents such as formamide. Hybridization specificity may be evaluated by comparison of hybridization to the test probes with hybridization to the various controls that can be present (*e.g.*, expression level control, normalization control, mismatch controls, *etc.*).

In general, there is a tradeoff between hybridization specificity (stringency) and signal intensity. Thus, in a preferred embodiment, the wash is performed at the highest stringency that produces consistent results and that provides a signal intensity greater than approximately 10% of the background intensity. Thus, in a preferred embodiment, the hybridized array may be washed at successively higher stringency solutions and read between each wash. Analysis of the data sets thus produced will reveal a wash stringency above which the hybridization pattern is not appreciably altered and which provides adequate signal for the particular oligonucleotide probes of interest.

### Signal Detection

The hybridized nucleic acids are typically detected by detecting one or more labels attached to the sample nucleic acids. The labels may be incorporated by any of a



number of means well known to those of skill in the art (see Lockhart *et al.*, (1999) WO 99/32660).

### Databases

The present invention includes relational databases containing sequence  
5 information, for instance for one or more of the genes of Table 1, as well as gene  
expression information in various liver tissue samples. Databases may also contain  
information associated with a given sequence or tissue sample such as descriptive  
information about the gene associated with the sequence information, descriptive  
information concerning the clinical status of the tissue sample, or information concerning  
10 the patient from which the sample was derived. The database may be designed to include  
different parts, for instance a sequence database and a gene expression database. The  
databases of the invention may be stored on any available computer-readable medium.  
Methods for the configuration and construction of such databases are widely available, for  
instance, see Akerblom *et al.*, (U.S. Patent 5,953,727), which is specifically incorporated  
15 herein by reference in its entirety.

The databases of the invention may be linked to an outside or external database.  
In a preferred embodiment, as described in Table 1, the external database is GenBank and  
the associated databases maintained by the National Center for Biotechnology Information  
or NCBI (<http://www.ncbi.nlm.nih.gov/Entrez/>). Other external databases that may be  
20 used in the invention include those provided by Chemical Abstracts Service  
(<http://stnweb.cas.org/>) and Incyte Genomics  
(<http://www.incyte.com/sequence/index.shtml>).

Any appropriate computer platform may be used to perform the necessary  
comparisons between sequence information, gene expression information and any other  
25 information in the database or provided as an input. For example, a large number of  
computer workstations are available from a variety of manufacturers, such as those  
available from Silicon Graphics. Client-server environments, database servers and  
networks are also widely available and appropriate platforms for the databases of the  
invention.

30 The databases of the invention may be used to produce, among other things,  
electronic Northern blots (E-Northern) to allow the user to determine the cell type or  
tissue in which a given gene is expressed and to allow determination of the abundance or

expression level of a given gene in a particular tissue or cell. The E-northern analysis can be used as a tool to discover tissue specific candidate therapeutic targets that are not over-expressed in tissues such as the liver, kidney, or heart. These tissue types often lead to detrimental side effects once drugs are developed and a first-pass screen to eliminate these targets early in the target discovery and validation process would be beneficial.

The databases of the invention may also be used to present information identifying the expression level in a tissue or cell of a set of genes comprising at least one gene in Table 1, comprising the step of comparing the expression level of at least one gene in Table 1 in the tissue to the level of expression of the gene in the database. Such methods may be used to predict the physiological state of a given tissue by comparing the level of expression of a gene or genes in Table 1 from a sample to the expression levels found in normal liver tissue, tissue from liver carcinomas or both. Such methods may also be used in the drug or agent screening assays as described herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The preceding working examples therefore, are illustrative only and should not be construed as limiting in any way the scope of the invention.

### **Examples**

#### ***Example 1: Preparation of Liver Disease Profiles***

##### ***Tissue Sample Acquisition and Preparation***

The patient tissue samples were derived from ten Korean patients, aged 34 to 65, and classified into two groups of five patients each. Each group contained samples from four men and one woman. One group consisted of patients who had been diagnosed with chronic viral hepatitis B and who later developed hepatic carcinomas. The second group of patients had been diagnosed with cirrhosis of the liver. These people also later developed hepatic carcinomas. For each patient, tissue was obtained from two areas of the liver to produce a set of biopsy samples. In the first patient group (cancer/hepatitis), samples were removed from liver tumors and from the non-cancerous surrounding area composed of inflamed tissue (inflammation due to hepatitis). In the second group

(cancer/cirrhosis), liver tissue was removed from tumors and from the non-cancerous surrounding area composed of fibrotic tissue (areas of fibrosis due to cirrhosis).

Histological analysis of each of the tissue samples was performed and samples were segregated into either non-cancerous or cancerous categories.

5 With minor modifications, the sample preparation protocol followed the Affymetrix GeneChip Expression Analysis Manual. Frozen tissue was first ground to powder using the Spex Certiprep 6800 Freezer Mill. Total RNA was then extracted using Trizol (Life Technologies). The total RNA yield for each sample (average tissue weight of 300 mg) was 200-500  $\mu$ g. Next, mRNA was isolated using the Oligotex mRNA Midi  
10 kit (Qiagen). Since the mRNA was eluted in a final volume of 400  $\mu$ l, an ethanol precipitation step was required to bring the concentration to 1  $\mu$ g/ $\mu$ l. Using 1-5  $\mu$ g of mRNA, double stranded cDNA was created using the SuperScript Choice system (Gibco-BRL). First strand cDNA synthesis was primed with a T7-(dT<sub>24</sub>) oligonucleotide. The cDNA was then phenol-chloroform extracted and ethanol precipitated to a final  
15 concentration of 1  $\mu$ g/ $\mu$ l.

From 2  $\mu$ g of cDNA, cRNA was synthesized according to standard procedures. To biotin label the cRNA, nucleotides Bio-11-CTP and Bio-16-UTP (Enzo Diagnostics) were added to the reaction. After a 37°C incubation for six hours, the labeled cRNA was cleaned up according to the Rneasy Mini kit protocol (Qiagen). The cRNA was then  
20 fragmented (5 $\times$  fragmentation buffer: 200 mM Tris-Acetate (pH 8.1), 500 mM KOAc, 150 mM MgOAc) for thirty-five minutes at 94°C.

55  $\mu$ g of fragmented cRNA was hybridized on the human and the Human Genome U95 set of arrays for twenty-four hours at 60 rpm in a 45°C hybridization oven. The chips were washed and stained with Streptavidin Phycoerythrin (SAPE) (Molecular Probes) in  
25 Affymetrix fluidics stations. To amplify staining, SAPE solution was added twice with an anti-streptavidin biotinylated antibody (Vector Laboratories) staining step in between. Hybridization to the probe arrays was detected by fluorometric scanning (Hewlett Packard Gene Array Scanner). Following hybridization and scanning, the microarray images were analyzed for quality control, looking for major chip defects or abnormalities in  
30 hybridization signal. After all chips passed QC, the data was analyzed using Affymetrix GeneChip software (v3.0), and Experimental Data Mining Tool (EDMT) software (v1.0).

### *Gene Expression Analysis*

All samples were prepared as described and hybridized onto the Affymetrix Human Genome U95 array. Each chip contains 16-20 oligonucleotide probe pairs per gene or cDNA clone. These probe pairs include perfectly matched sets and mismatched sets, both of which are necessary for the calculation of the average difference. The average difference is a measure of the intensity difference for each probe pair, calculated by subtracting the intensity of the mismatch from the intensity of the perfect match. This takes into consideration variability in hybridization among probe pairs and other hybridization artifacts that could affect the fluorescence intensities. Using the average difference value that has been calculated, an absolute call for each gene is made.

The absolute call of present, absent or marginal is used to generate a Gene Signature, a tool used to identify those genes that are commonly present or commonly absent in a given sample set, according to the absolute call.

The Gene Signature Curve is a graphic view of the number of genes consistently present in a given set of samples as the sample size increases, taking into account the genes commonly expressed among a particular set of samples, and discounting those genes whose expression is variable among those samples. The curve is also indicative of the number of samples necessary to generate an accurate Gene Signature. As the sample number increases, the number of genes common to the sample set decreases. The curve is generated using the positive Gene Signatures of the samples in question, determined by adding one sample at a time to the Gene Signature, beginning with the sample with the smallest number of present genes and adding samples in ascending order. The curve displays the sample size required for the most consistency and the least amount of expression variability from sample to sample. The point where this curve begins to level off represents the minimum number of samples required for the Gene Signature. Graphed on the x-axis is the number of samples in the set, and on the y-axis is the number of genes in the positive Gene Signature. As a general rule, the acceptable percent of variability in the number of positive genes between two sample sets should be less than 5%.

For the purposes of this study, the following statistical methods were used for the data analysis. A gene set consists of genes that have a certain percentage of present calls in at least one group of samples. These genes are analyzed, and others are excluded. For example, a gene having 40% present calls (2 out of 5 samples) in at least in one sample group, cancerous cells from either hepatitis or cirrhosis patients, or non-cancerous cells

from either type of patient, is included in the analysis if 40% is above the lower limit for percent present calls. Also, the genes are divided into two groups depending on their expression values across samples. For the genes in the high expression group, the average difference value is transformed to log scale before the analysis. For the genes in the low expression group, the original values are used in the analysis. An Analysis of Variance (ANOVA) method is used for data analysis (Steel et al., Principles and Procedures of Statistics: A Biometrical Approach, Third Ed., McGraw-Hill, 1997). Prior to the final analysis, a leave-one-out approach is used for outlier detection. One sample is left out of the ANOVA analysis to see whether omitting a specific sample from the analysis has any significant effect on the final result. If so, that particular sample is excluded from the final analysis. After outlier detection, the final analysis produces a list of genes that are differentially expressed with a p-value  $\leq 0.001$  as determined by the contrast from the ANOVA.

Differentially expressed genes were discovered by comparing biopsy samples from cancerous and non-cancerous regions of the same liver in patients with chronic viral hepatitis (CH) or liver cirrhosis (LC) who went on to develop primary liver cancer (hepatocellular carcinoma or HCC). Genes which showed no difference in expression level between a the cancerous and non-cancerous samples were not included in Table 1. Group 1 of Table 1 (23 genes) lists the genes that were found to be differentially expressed when the level in liver tumor cells was compared to the level in non-cancerous cells from inflamed areas or from fibrotic areas. Group 2 (12 genes) lists the genes whose expression level differed in liver tumor cells compared to cells from areas of inflammation, and group 3 contains those genes whose expression level differed in liver tumor cells compared to cells from fibrotic regions of the liver (74 genes).

#### ***Fold Change analysis***

The data was first filtered to exclude all genes that showed no expression in any of the samples. The ratio (cancerous/non-cancerous, HCC/CH or HCC/LC) was calculated by comparing the mean expression value for each gene in the cancerous sample set against the mean expression value of that gene in the non-cancerous sample set. Genes were included in the analysis if they had a fold change  $\geq 1.5$  in either direction, and a p-value  $< 0.0007$  as determined by an Analysis of Variance Test (ANOVA). According to the criteria of the test, differences having p-values below 0.0007 were determined to be

statistically significant. Out of the ~60,000 genes surveyed by the Human Genome U95 set, 109 genes were present in the overall fold change analysis. In Table 1, numbers representing a comparison, or fold change, between the level of expression of a gene in two disease state liver biopsy samples can be positive or negative. Positive values indicate a higher expression level in the cancerous sample compared to the non-cancerous sample (up-regulation), while negative values indicate a lower expression level in the cancerous sample compared to the non-cancerous sample (down-regulation).

### ***Expression Profiles of Genes Differentially Expressed in Liver Disease***

Using the above described methods, genes that were predominantly over-expressed in liver cancer, or predominantly under-expressed in liver cancer, were identified. Genes with consistent differential expression patterns provide potential targets for broad range diagnostics and therapeutics.

Table 1 lists the genes determined to be differentially expressed in cancerous liver tissue compared to non-cancerous liver tissue, with the fold change value for each gene. More specifically, the level of expression of the genes of Table 1 in liver cancer cells was compared to the level of expression in tissue from inflamed and/or fibrotic areas of the liver. The set of genes in each group, along with their relative expression levels, creates a profile for the diseases examined, chronic hepatitis with hepatic carcinoma and cirrhosis with hepatic carcinoma.

These genes or subsets of these genes confirm an overall liver disease gene expression profile. The genes in Table 1 may be used alone, or in combination with the methods, compositions, databases and computer systems of the invention.

### ***Example 2: Diagnostic Subset of Liver Disease Associated GeneCluster Analysis***

Table 1 lists the members of diagnostic subsets of genes selected by p-value in groups 2 and 3 (12 and 74 genes, respectively). In addition to their diagnostic, monitoring, drug screening and therapeutic uses, these groups of genes can be used to differentiate between liver tumor samples from subjects with chronic hepatitis and liver tumor samples from subjects with cirrhosis. Assays measuring the expression level of these genes are capable of distinguishing between carcinomas arising in chronic hepatitis patients versus carcinomas arising in cirrhosis patients.

The gene subsets of Table 1 can, therefore, be used to identify the presence of a malignant tumor in liver tissue from chronic hepatitis or cirrhosis patients, to monitor the progression of the tumor (*e.g.*, during cancer treatment or combined disease treatments), to evaluate the effects of therapeutic agents for treating the tumor or to distinguish the origin or predisposing condition of the tumor.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims. All cited patents and publications referred to in this application are herein incorporated by reference in their entirety.

Table 1- Genes Differentially Expressed in Liver Cancer

Fragment Name	Seq. ID	Accession Number	UniGene ID	Description	p-Values for			
					HCC/CH	HCC/LC	HCC/LC	
Group 1 : HCC/CH and HCC/LC								
33428_s_at	1	AF034957	Hs.194019	atractin	4.10	2.84	0.0002690	0.0002720
36785_at	2	Z23090	Hs.76067	heat shock 27kD protein 1	2.71	3.49	0.0001100	0.0000150
74893_g_at	3	AA928646	Hs.75864	endoplasmic reticulum glycoprotein	2.47	2.25	0.000748	0.000034
51788_at	4	AW023096	Hs.3887	proteasome (prosome, macropain) 26S subunit, non-ATPase, 1	2.03	1.74	0.000464	0.000647
44143_at	5	AA399076	Hs.46743	McKusick-Kaufman syndrome	1.65	1.52	0.000645	0.00038
832_at	6	U39317	Hs.108332	ubiquitin-conjugating enzyme E2D 2 (homologous to yeast UBC4/5)	1.50	1.69	0.000222	0.000274
57042_at	7	W74749	Hs.285818	similar to Caenorhabditis elegans protein C42C1.9	-1.80	-2.71	0.000394	0.000056
55107_at	8	A1916306	Hs.87125	EH-domain containing 3	-1.91	-3.32	0.000099	0
33766_at	9	X77777	Hs.198726	vasoactive intestinal peptide receptor 1	-1.96	-2.43	0.000039	0.00001
37206_at	10	X63359	Hs.294039	UDP glycosyltransferase 2 family, polypeptide B10	-2.03	-4.69	0.000016	4.00E-06
37059_at	11	Z48475	Hs.89771	glucokinase (hexokinase 4) regulatory protein	-2.41	-10.31	0.000679	0.000872
533_g_at	12	U17418	Hs.1019	parathyroid hormone receptor 1	-2.77	-3.31	0.000064	0.00003
35803_at	13	S82240	Hs.6838	ras homolog gene family, member E	-3.62	-8.20	1.00E-06	0
33862_at	14	AF017786	Hs.173717	phosphatidic acid phosphatase type 2B	-4.27	-3.55	0.000161	0.000757
44982_s_at	15	A1985046	Hs.24395	small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAX)	-5.65	-12.23	0	0
32666_at	16	U19495	Hs.237356	stromal cell-derived factor 1	-6.47	-6.54	0.000041	0.000012
35118_at	17	M12625	Hs.325507	lecithin-cholesterol acyltransferase	-6.48	-23.56	0.000032	0.000165
55063_at	18	AL042399	Hs.75668	glutamate decarboxylase 1 (brain, 67kD)	-7.52	-23.54	0.000099	7.00E-06
34602_at	19	D63160	Hs.54517	ficollin (collagen/fibrinogen domain-containing lectin) 2 (hucolin)	-7.54	-10.65	0.000032	0.00002
34708_at	20	D88587	Hs.333383	ficollin (collagen/fibrinogen domain-containing) 3 (Hakata antigen)	-9.43	-21.71	0.000271	0.000059
39120_at	21	AA224832	Hs.94360	metallothionein 1L	-9.97	-35.38	0.000089	3.00E-06
45943_at	22	A1052592	Hs.35718	cytochrome P450, subfamily VIIIB (sterol 12-alpha-hydroxylase), polypeptide 1	-11.93	-26.66	0.000178	0.000914
56641_at	23	A1937227	Hs.8821	liver-expressed antimicrobial peptide	-19.90	-66.71	0.000708	0.00097
Group 2 : HCC/CH								
45313_at	24	AA167715	Hs.296244	fatty acid synthase	4.22	2.44	0.000396	.
85972_at	25	A1424433	Hs.306000	solute carrier family 4 (anion exchanger), member 1, adapter protein	1.92	2.24	0.000318	.
1840_g_at		HG1112-HT11	Hs.10842	RAN, member RAS oncogene family	1.96	1.88	0.000705	.
33667_at	26	X52851	Hs.182937	peptidylprolyl isomerase A (cyclophilin A)	1.74	1.35	0.000879	.
53474_at	27	AF072812	Hs.7765	chromosome 16 open reading frame 5	-1.91	-2.04	0.000253	.



Table 1- Genes Differentially Expressed in Liver Cancer

Fragment Name	Seq. ID	Accession Number	UniGene ID	Description	HCC/CH	HCC/LC	p-Values for	
							HCC/CH	HCC/LC
34367_at	28	AF060643	Hs.3343	phosphoglycerate dehydrogenase	-4.06	-2.72	0.000976	.
38862_at	29	Y11215	Hs.19126	src kinase-associated phosphoprotein of 55 kDa	-3.73	-3.09	0.000593	.
40325_at	30	AB014460	Hs.66196	nth (E.coli endonuclease III)-like 1	-2.52	-3.18	0.000312	.
32727_at	31	AF037062	Hs.172914	retinol dehydrogenase 5 (11-cis and 9-cis)	-4.50	-3.42	0.000216	.
37319_at	32	M35878	Hs.77326	insulin-like growth factor binding protein 3	-5.33	-3.71	0.000339	.
35063_at	33	D50030	Hs.104	HGF activator	-3.99	-8.29	0.000159	.
1391_s_at	34	L04751	Hs.1645	cytochrome P450, subfamily IVA, polypeptide 11	-1.76	-14.90	0.000824	.
32966_at	35	L27050	Hs.2388	apolipoprotein F	-6.33	-16.30	0.000026	.
<b>Group 3 : HCC/LC</b>								
37482_at	36	U37100	Hs.116724	aldo-keto reductase family 1, member B11 (aldose reductase-like)	11.79	27.19	.	0.000874
33404_at	37	U02390	Hs.296341	adenylyl cyclase-associated protein 2	2.60	7.77	.	0.0002
63545_at	38	AW006831	Hs.337478	RAB, member of RAS oncogene family-like 2B	3.71	7.20	.	0.000793
34390_at	39	U90441	Hs.3622	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide II	3.58	6.27	.	0.000211
33873_at	40	D43642	Hs.2430	transcription factor-like 1	2.29	5.88	.	0.000472
893_at	41	M91670	Hs.174070	ubiquitin carrier protein	2.51	5.85	.	0.000206
59749_at	42	A1478190	Hs.324178	solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 6	1.21	5.18	.	0.000638
39749_at	43	U51007	Hs.148495	proteasome (prosome, macropain) 26S subunit, non-ATPase, 4	2.01	4.91	.	0.000288
44695_at	44	A1953020	Hs.324618	HSPC142 protein	2.71	4.66	.	0.000153
43836_s_at	45	A1971969	Hs.282997	glucosidase, beta; acid (includes glucosylceramidase)	1.91	4.21	.	9.00E-06
39801_at	46	AF046889	Hs.153357	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	3.32	4.11	.	0.000257
44219_at	47	A1937030	Hs.287883	X111-binding protein 51	1.44	3.92	.	0.00032
37256_at	48	A1829890	Hs.78524	TcD37 homolog	1.79	3.76	.	0.00064
37399_at	49	D17793	Hs.78183	aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)	2.53	3.76	.	4.00E-06
35820_at	50	X62078	Hs.289082	GM2 ganglioside activator protein	2.10	3.69	.	0.00034
1100_at	51	L76191	Hs.182018	interleukin-1 receptor-associated kinase 1	3.76	3.60	.	0.000816
146_at	52	U81802	Hs.154846	phosphatidylinositol 4-kinase, catalytic, beta polypeptide	1.90	3.42	.	0.000767
77990_at	53	AW004018	Hs.268281	CGI-201 protein	1.76	3.39	.	0.000842

Table 1- Genes Differentially Expressed in Liver Cancer

Fragment Name	Seq. ID	Accession Number	UniGene ID	Description	HCC/CH	HCC/LC	p-Values for	
							HCC/CH	HCC/LC
32799_at	54	AF023268	Hs.200600	secretory carrier membrane protein 3	1.81	3.25	.	0.00009
32260_at	55	X86809	Hs.194673	phosphoprotein enriched in astrocytes 15	2.14	3.17	.	0.000599
497_at	56	U32680	Hs.194660	ceroid-lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeier-Vogt disease)	1.97	3.14	.	0.00013
33154_at	57	D26800	Hs.89545	proteasome (prosome, macropain) subunit, beta type, 4	1.62	3.08	.	0.000019
39062_at	58	AL008726	Hs.118126	protective protein for beta-galactosidase (galactosialidosis)	2.18	3.05	.	0.00004
56378_at	59	W22366	Hs.337078	NICE-5 protein	1.45	3.02	.	0.000292
45155_at	60	AI433892	Hs.38738	claudin 15	2.14	2.95	.	0.000669
44082_at	61	AA029831	Hs.238928	HT002 protein; hypertension-related calcium-regulated gene	1.63	2.94	.	0.00064
57136_at	62	AI279571	Hs.23528	HSPC038 protein	1.54	2.93	.	0.000293
64501_at	63	AI982714	Hs.93832	putative membrane protein	1.97	2.70	.	0.000498
34835_at	64	D87442	Hs.4788	nicastatin	1.62	2.69	.	0.000332
41322_s_at	65	AI816034	Hs.23990	nucleolar protein family A, member 2 (H1ACA small nucleolar RNPs)	1.89	2.61	.	0.000756
44821_at	66	AI634570	Hs.301005	purine-rich element binding protein B	1.96	2.58	.	0.000034
48913_at	67	AI023344	Hs.12865	p47	1.51	2.56	.	0.000807
35685_at	68	Z14000	Hs.35384	ring finger protein 1	1.43	2.52	.	0.000061
64886_at	69	AA632300	Hs.65648	RNA binding motif protein 8A	1.58	2.40	.	0.000325
74577_s_at	70	AI798743	Hs.183994	RAD9 (S. pombe) homolog	1.77	2.38	.	0.000159
45712_at	71	H98166	Hs.279868	SUMO-1 activating enzyme subunit 1	1.52	2.30	.	0.000214
90637_at	72	AA039699	Hs.7101	anaphase-promoting complex subunit 5	1.40	2.28	.	0.000854
1659_s_at	73	D78132	Hs.279903	Ras homolog enriched in brain 2	1.98	2.20	.	0.00049
38719_at	74	U03985	Hs.108802	N-ethylmaleimide-sensitive factor	2.03	2.14	.	0.000546
37669_s_at	75	U16799	Hs.78629	ATPase, Na+/K+ transporting, beta 1 polypeptide	1.58	2.12	.	0.000082
45255_at	76	AI354351	Hs.237924	CGI-69 protein	1.99	2.11	.	0.000599
1309_at	77	D26598	Hs.82793	proteasome (prosome, macropain) subunit, beta type, 3	1.91	1.83	.	0.000106
33659_at	78	X95404	Hs.180370	cofilin 1 (non-muscle)	1.64	1.63	.	0.000997
35752_s_at	79	M15036	Hs.64016	protein S (alpha)	-1.42	-2.03	.	0.000865
64369_s_at	80	AA219354	Hs.282804	ceruloplasmin (ferroxidase)	-1.79	-2.31	.	0.000802
260_at	81	M16447	Hs.75438	quinoid dihydropteridine reductase	-1.76	-2.65	.	0.000586
40082_at	82	D10040	Hs.154890	fatty-acid-Coenzyme A ligase, long-chain 2	-1.26	-2.98	.	0.000059
46746_s_at	83	W42636	Hs.5326	porcupine	-1.34	-3.05	.	0.000074

Table 1- Genes Differentially Expressed in Liver Cancer

Fragment Name	Seq. ID	Accession Number	UniGene ID	Description	HCC/CH	HCC/LC	p-Values for	
							HCC/CH	HCC/LC
90033_at	84	T66157	Hs.154437	phosphodiesterase 2A, cGMP-stimulated	-2.22	-3.41	.	0.000467
36097_at	85	M62831	Hs.737	immediate early protein	-1.64	-4.02	.	0.000036
74184_at	86	T98839	Hs.30299	IGF-II mRNA-binding protein 2	-1.48	-4.78	.	0.000181
37022_at	87	U41344	Hs.76494	proline arginine-rich end leucine-rich repeat protein	-2.63	-4.89	.	0.000047
58322_at	88	A1765890	Hs.16341	MAWD binding protein	-1.45	-5.43	.	0.000166
65867_at	89	AL043089	Hs.3807	FXYD domain-containing ion transport regulator 6	-1.70	-5.44	.	0.000143
38634_at	90	M11433	Hs.101850	retinol-binding protein 1, cellular	-6.44	-5.51	.	0.000016
38772_at	91	Y11307	Hs.8867	cysteine-rich, angiogenic inducer, 61	-4.09	-5.72	.	0.000487
46694_at	92	A1078144	Hs.9315	HNOEL-iso protein	-4.66	-5.88	.	0.000875
48502_at	93	AA122235	Hs.113052	RNA cyclase homolog	-3.30	-6.27	.	0.000765
64390_at	94	A1342377	Hs.44281	CDK4-binding protein p34SE1	-2.10	-6.59	.	0.000087
1212_at	95	U86529	Hs.26403	glutathione transferase zeta 1 (maleylacetate isomerase)	-3.51	-7.30	.	0.000367
42363_r_at	96	A1680350	Hs.296176	STAT induced STAT inhibitor 3	-1.71	-7.37	.	0.000016
91311_at	97	AA576961	Hs.82101	pleckstrin homology-like domain, family A, member 1	-2.77	-7.52	.	0.000083
37972_at	98	U75744	Hs.88646	deoxyribonuclease I-like 3	-3.21	-7.76	.	0.000691
1379_at	99	M59371	Hs.171596	epithelial receptor protein-tyrosine kinase	-1.67	-7.95	.	0
35925_at	100	AF040639	Hs.284236	aldo-keto reductase family 7, member A3 (aflatoxin aldehyde reductase)	-2.65	-9.29	.	0.000105
34638_r_at	101	M12963	Hs.73843	alcohol dehydrogenase 1 (class I), alpha polypeptide	-1.85	-9.89	.	0.00081
				coagulation factor IX (plasma thromboplastic component, Christmas disease, hemophilia B)	-1.84	-10.42	.	0.00065
35556_at	102	K02402	Hs.1330	UDP glycosyltransferase 2 family, polypeptide B7	-3.64	-10.49	.	0.000071
41376_i_at	103	J05428	Hs.10319	L-kynurenine/alpha-aminoadipate aminotransferase	-3.46	-10.90	.	0.000621
61370_at	104	A1819354	Hs.301528	afamin	-1.72	-11.83	.	0.000065
33564_at	105	L32140	Hs.531	metallothionein 1F (functional)	-3.56	-11.84	.	0.000364
31622_f_at	106	M10943	Hs.203936	alcohol dehydrogenase 2 (class I), beta polypeptide	-4.24	-13.10	.	0.000706
35730_at	107	X03350	Hs.4	complement component 7	-3.50	-15.87	.	0.000247
37394_at	108	J03507	Hs.78065	metallothionein 1A (functional)	-5.12	-32.78	.	0.000409
31623_f_at	109	K01383	Hs.173451					

Table 1- Genes Differentially Expressed in Liver Cancer

Fragment Name	Seq. ID	CH/LC	HCC(CH) / HCC(LC)	Mean(CH)	Mean(HCC from CH)	Mean(LC)	Mean(HCC from LC)
<b>Group 1 : HCC/CH and HCC/LC</b>							
33428_s_at	1	-1.35	1.06	36.99	151.48	50.05	142.36
36785_at	2	1.15	-1.12	1300.25	3526.56	1133.51	3956.77
74893_g_at	3	-1.19	-1.09	1381.07	3404.95	1647.94	3710.79
51788_at	4	-1.36	-1.17	725.34	1470.84	987.9	1714.14
44143_at	5	-1.38	-1.27	193.1	319.1	265.76	404.33
832_at	6	1.06	-1.06	151.86	227.89	143.41	242.01
57042_at	7	-1.46	1.03	875.09	485.4	1278.17	472.25
55107_at	8	-1.31	1.33	387.69	203.24	506.63	152.43
33766_at	9	-1.24	1.00	39.13	20	48.58	20
37206_at	10	-2.07	1.12	386.63	190.39	799.12	170.46
37059_at	11	-1.25	3.43	258.26	107.34	322.94	31.33
533_g_at	12	1.54	1.84	192.89	69.68	125.26	37.81
35803_at	13	-2.03	1.11	211.11	58.27	429.09	52.34
33862_at	14	1.00	-1.20	1039.82	243.42	1035.68	291.65
44982_s_at	15	-1.60	1.35	152.67	27.03	244.6	20
32666_at	16	-1.15	-1.14	206.74	31.94	238.32	36.44
35118_at	17	-1.55	2.34	469.05	72.38	728.59	30.93
55063_at	18	-2.08	1.50	494.25	65.7	1028.8	43.7
34602_at	19	-1.33	1.06	260.1	34.51	345.97	32.48
34708_at	20	-1.76	1.31	761.37	80.76	1337.08	61.6
39120_at	21	-2.27	1.56	2259.52	226.61	5124.11	144.85
45943_at	22	-2.46	-1.10	1674.55	140.37	4117.95	154.45
56641_at	23	-3.95	-1.18	4054.26	203.69	16004.11	239.9
<b>Group 2 : HCC/CH</b>							
45313_at	24	-1.53	1.13	158.25	667.72	241.75	590.69
85972_at	25	-1.56	-1.82	24.11	46.33	37.62	84.41
1840_g_at	26	-1.10	-1.06	353.18	691.4	388.94	730.83
33667_at	26	-1.11	1.15	2848.2	4949.09	3175.61	4297.99
53474_at	27	-1.62	-1.52	246.2	128.65	398.43	195.14

Table 1- Genes Differentially Expressed in Liver Cancer

Fragment Name	Seq. ID	CH/LC	HCC(CH) / HCC(LC)	Mean(CH)	Mean(HCC from CH)	Mean(LC)	Mean(HCC from LC)
34367_at	28	-1.04	-1.55	605.74	149.24	630.02	231.53
38862_at	29	-1.14	-1.38	93.76	25.12	106.98	34.66
40325_at	30	1.14	1.43	211	83.67	185.74	58.46
32727_at	31	1.17	-1.12	89.92	20	77.01	22.5
37319_at	32	-1.02	-1.46	2159.87	404.88	2196.02	591.49
35063_at	33	-1.25	1.66	423.71	106.19	529.69	63.87
1391_s_at	34	-1.48	5.71	1096.07	622.87	1625.85	109.13
32966_at	35	-1.42	1.82	371.31	58.66	525.9	32.26
<b>Group 3 : HCC/LC</b>							
37482_at	36	-2.68	-6.18	30.34	357.74	81.37	2212.61
33404_at	37	1.27	-2.36	25.3	65.78	20	155.35
63545_at	38	1.15	-1.68	52.84	196.23	45.81	329.84
34390_at	39	-1.12	-1.95	22.56	80.76	25.18	157.82
33873_at	40	1.39	-1.84	83.99	192.42	60.26	354.05
893_at	41	1.49	-1.56	58.78	147.44	39.34	230.06
59749_at	42	2.80	-1.53	100.08	120.75	35.71	184.96
39749_at	43	1.04	-2.34	108.86	219.04	104.32	511.93
44695_at	44	1.71	-1.01	34.2	92.53	20	93.12
43836_s_at	45	1.15	-1.92	484.14	924.31	422.21	1775.62
39801_at	46	1.15	-1.08	157.64	524.07	137.41	564.88
44219_at	47	1.32	-2.06	173.41	250.32	131.61	515.98
37256_at	48	1.32	-1.59	46.64	83.47	35.25	132.39
37399_at	49	-1.23	-1.83	642.62	1628.75	791.81	2974.46
35820_at	50	1.78	1.01	91.39	192.04	51.31	189.52
1100_at	51	-1.07	-1.03	41.77	157.11	44.84	161.26
146_at	52	1.11	-1.61	45.26	86.08	40.62	138.85
77990_at	53	1.31	-1.47	92.53	163.29	70.82	240.01

Table 1- Genes Differentially Expressed in Liver Cancer

Fragment Name	Seq. ID	CH/LC	HCC(CH) / HCC(LC)	Mean(CH)	Mean(HCC from CH)	Mean(LC)	Mean(HCC from LC)
32799_at	54	1.13	-1.59	201.78	365.98	179.36	582.69
32260_at	55	1.17	-1.26	215.21	460.32	183.36	581.74
497_at	56	1.04	-1.53	183.55	323.01	157.23	493.58
33154_at	57	-1.01	-1.92	476.51	771.16	481.66	1481.48
39062_at	58	-1.01	-1.42	323.59	705.43	328.02	1000.16
56378_at	59	1.10	-1.89	586.6	847.94	530.86	1603.61
45155_at	60	1.39	1.01	509.58	1088.23	367.1	1081.19
44082_at	61	1.05	-1.71	145.39	237.67	138.47	407.05
57136_at	62	-1.02	-1.94	856.14	1320.28	873.09	2557.79
64501_at	63	-1.16	-1.59	939.04	1846.8	1086.52	2938.37
34835_at	64	1.02	-1.63	289.56	469.17	284.43	766.35
41322_s_at	65	1.03	-1.34	84.42	159.51	81.92	213.73
44821_at	66	-1.08	-1.43	389.21	762.25	421.21	1088.69
48913_at	67	1.06	-1.60	380.42	575.38	358.46	918.11
35685_at	68	1.64	-1.07	289.35	413.86	176.34	443.67
64886_at	69	-1.48	-2.25	442.13	697.6	654	1568.34
74577_s_at	70	-1.07	-1.44	1553.52	2748.37	1669.16	3966.04
45712_at	71	-1.01	-1.53	336.68	512.07	340.41	782.95
90637_at	72	1.41	-1.15	216.23	302.5	153.02	348.45
1659_s_at	73	1.15	1.04	285.54	526.93	230.24	506.23
38719_at	74	-1.11	-1.17	56.48	114.7	62.54	134.02
37669_s_at	75	-1.03	-1.38	1005.93	1588.92	1034.68	2197.29
45255_at	76	1.15	1.09	1106.18	2205.3	960.49	2029
1309_at	77	-1.20	-1.15	416.77	795.57	500.68	914.19
33659_at	78	-1.00	1.01	1604.43	2635.01	1605.92	2620.97
35752_s_at	79	-1.06	1.34	266.49	187.67	283.7	139.98
64369_s_at	80	-1.23	1.05	1096.17	613.12	1345.19	582.06
260_at	81	-1.50	1.00	330.36	187.35	494.66	186.66
40082_at	82	-2.20	1.07	456.38	360.83	1003.4	336.73
46746_s_at	83	-1.96	1.16	889.16	664.38	1739.7	571.32

Table 1- Genes Differentially Expressed in Liver Cancer

Fragment Name	Seq. ID	CH/LC	HCC(CH) / HCC(LC)	Mean(CH)	Mean(HCC from CH)	Mean(LC)	Mean(HCC from LC)
90033_at	84	-1.09	1.41	596.98	268.41	648.03	190.08
36097_at	85	-1.17	2.09	860.22	522.96	1004.81	249.96
74184_at	86	-2.48	1.30	159.53	107.88	395.36	82.7
37022_at	87	1.25	2.33	315.21	119.91	251.61	51.46
58322_at	88	-2.17	1.72	2508.42	1725.07	5453.68	1004.8
65867_at	89	1.21	3.87	2602.05	1527.55	2143.83	394.41
38634_at	90	1.50	1.28	682.93	106.06	455.03	82.61
38772_at	91	1.12	1.56	193.88	47.36	173.31	30.31
46694_at	92	1.99	2.52	322.34	69.24	161.78	27.53
48502_at	93	-1.64	1.16	935.83	283.76	1535.68	245.08
64390_at	94	-1.68	1.88	269.34	128.48	451.21	68.52
1212_at	95	1.06	2.20	374.12	106.47	352.4	48.29
42363_r_at	96	-3.60	1.19	851	496.58	3065.8	416.23
91311_at	97	-2.98	-1.10	334.15	120.6	994.75	132.35
37972_at	98	-1.34	1.80	450.49	140.24	604.69	77.9
1379_at	99	-3.28	1.45	48.46	29	158.9	20
35925_at	100	-1.96	1.79	163.57	61.72	320.94	34.54
34638_r_at	101	-2.60	2.06	351.38	189.82	912.75	92.25
35556_at	102	-2.34	2.42	621.13	337.88	1452.17	139.35
41376_i_at	103	-2.77	1.04	1104.52	303.33	3061.38	291.7
61370_at	104	-1.76	1.79	161.16	46.57	283.6	26.03
33564_at	105	-2.99	2.29	237.07	137.46	708.95	59.9
31622_f_at	106	-1.89	1.76	3587.58	1009.05	6782.49	572.84
35730_at	107	-1.82	1.69	539.69	127.19	982.94	75.05
37394_at	108	-1.44	3.15	506.19	144.63	728.66	45.9
31623_f_at	109	-1.93	3.31	3516.77	686.61	6798.83	207.38

Table 2: Patient Information

Sample ID	Donor Gender	Donor Race	Donor Age at Excision	Date of Collection	Organ/Fluid	Tissue Site	Normal or Diseased	Specimen Diagnosis
YUMC-034-01	Male	Korean	42	4/2/2001	Liver	left lobe	Diseased	Liver cirrhosis (HBV)
YUMC-034-02	Male	Korean	42	4/2/2001	Liver	left lobe	Malignant	Hepatoma
YUMC-035-01	Male	Korean	34	1/29/2001	Liver	right lobe	Diseased	Chronic hepatitis B
YUMC-035-02	Male	Korean	34	1/29/2001	Liver	right lobe	Malignant	Hepatoma
YUMC-036-01	Female	Korean	43	2/16/2001	Liver	left lobe	Diseased	Liver cirrhosis (HBV)
YUMC-036-02	Female	Korean	43	2/16/2001	Liver	left lobe	Malignant	Hepatoma
YUMC-037-01	Female	Korean	65	2/14/2001	Liver	right lobe	Diseased	Chronic hepatitis B
YUMC-037-02	Female	Korean	65	2/14/2001	Liver	right lobe	Malignant	Hepatoma
YUMC-038-01	Male	Korean	37	2/21/2001	Liver	right lobe	Diseased	Liver cirrhosis (HBV)
YUMC-038-02	Male	Korean	37	2/21/2001	Liver	right lobe	Malignant	Hepatoma
YUMC-039-01	Male	Korean	62	4/5/2001	Liver	right lobe	Diseased	Liver cirrhosis (HBV)
YUMC-039-02	Male	Korean	62	4/5/2001	Liver	right lobe	Malignant	Hepatoma
YUMC-040-01	Male	Korean	40	3/30/2001	Liver	right lobe	Diseased	Liver cirrhosis (HBV)
YUMC-040-02	Male	Korean	40	3/30/2001	Liver	right lobe	Malignant	Hepatoma
YUMC-042-01	Male	Korean	61	12/18/2000	Liver	left lobe	Diseased	Chronic hepatitis B
YUMC-042-02	Male	Korean	61	12/18/2000	Liver	left lobe	Malignant	Hepatoma
YUMC-043-01	Male	Korean	63	3/27/2001	Liver	left lobe	Diseased	Chronic hepatitis B
YUMC-043-02	Male	Korean	63	3/27/2001	Liver	left lobe	Malignant	Hepatoma
YUMC-059-01	Male	Korean	62	3/26/2001	Liver	right lobe	Diseased	chronic hepatitis B
YUMC-059-02	Male	Korean	62	3/26/2001	Liver	right lobe	Malignant	hepatocellular carcinoma



What is claimed is:

1. A method of diagnosing liver cancer in a patient, comprising:
  - (a) detecting the level of expression in a tissue sample of one or more genes from Table 1; wherein differential expression of the genes in Table 1 is indicative of liver cancer.
2. A method of detecting the progression of liver cancer in a patient, comprising:
  - (a) detecting the level of expression in a tissue sample of one or more genes from Table 1; wherein differential expression of the genes in Table 1 is indicative of liver cancer progression.
3. A method of monitoring the treatment of a patient with liver cancer, comprising:
  - (a) administering a pharmaceutical composition to the patient;
  - (b) preparing a gene expression profile of one or more of the genes in Table 1 from a cell or tissue sample from the patient; and
  - (c) comparing the patient gene expression profile to a gene expression profile from a cell population selected from the group consisting of non-cancerous liver cells and cancerous liver cells.
4. A method of treating a patient with liver cancer, comprising:
  - (a) administering to the patient a pharmaceutical composition;
  - (b) preparing a gene expression profile of one or more of the genes in Table 1 from a cell or tissue sample from the patient; and
  - (c) comparing the patient expression profile to a gene expression profile selected from the group consisting of non-cancerous liver cells and cancerous liver cells.
5. A method of typing liver disease in a patient, comprising:
  - (a) detecting the level of expression in a tissue sample of one or more genes from Table 1; wherein differential expression of the genes in Table 1 is indicative of a type of liver disease selected from a group consisting of chronic hepatitis with hepatic carcinoma and cirrhosis with hepatic carcinoma.
6. A method of detecting the presence or progression of liver cancer in a patient with chronic hepatitis, comprising:

(a) detecting the level of expression in a tissue sample of one or more genes from Table 1; wherein differential expression of the genes in Table 1 is indicative of chronic hepatitis with liver cancer.

5 7. A method of detecting the presence or progression of liver cancer in a patient with cirrhosis, comprising:

(a) detecting the level of expression in a tissue sample of one or more genes from Table 1; wherein differential expression of the genes in Table 1 is indicative of cirrhosis with liver cancer.

10 8. A method of diagnosing liver cancer according to claim 1, wherein the liver cancer is accompanied by chronic hepatitis or cirrhosis.

9. A method of differentiating liver cancer related to chronic hepatitis from liver cancer related to cirrhosis in a patient, comprising:

15 (a) detecting the level of expression in a tissue sample of one or more genes from Table 1; wherein differential expression of the genes in Table 1 is indicative of either liver cancer related to chronic hepatitis or liver cancer related to cirrhosis.

10. A method of screening for an agent capable of modulating the onset or progression of liver cancer, comprising:

20 (a) preparing a first gene expression profile of a cell population comprising cancerous liver cells, wherein the expression profile comprises the expression level of one or more genes from Table 1;

(b) exposing the cell population to the agent;

(c) preparing second gene expression profile of the agent-exposed cell population; and

(d) comparing the first and second gene expression profiles.

25 11. The method of claim 10, wherein the liver cancer is chronic hepatitis with liver cancer.

12. The method of claim 10, wherein the liver disease is cirrhosis with liver cancer.

13. A composition comprising at least two oligonucleotides, wherein each of the oligonucleotides comprises a sequence that specifically hybridizes to a gene in Table 1.
14. A composition according to claim 13, wherein the composition comprises at least 3 oligonucleotides.
- 5 15. A composition according to claim 13, wherein the composition comprises at least 5 oligonucleotides.
16. A composition according to claim 13, wherein the composition comprises at least 7 oligonucleotides.
17. A composition according to claim 13, wherein the composition comprises at least  
10 10 oligonucleotides.
18. A composition according to any one of claims 13-17, wherein the oligonucleotides are attached to a solid support.
19. A composition according to claim 18, wherein the solid support is selected from a group consisting of a membrane, a glass support, a filter, a tissue culture dish, a polymeric  
15 material, a bead and a silica support.
20. A solid support comprising at least two oligonucleotides, wherein each of the oligonucleotides comprises a sequence that specifically hybridizes to a gene in Table 1.
21. A solid support according to claim 20, wherein the oligonucleotides are covalently  
20 attached to the solid support.
22. A solid support according to claim 20, wherein the oligonucleotides are non-covalently attached to the solid support.
23. A solid support according to claim 20, wherein the support comprises at least about 10 different oligonucleotides in discrete locations per square centimeter.
- 25 24. A solid support according to claim 20, wherein the support comprises at least about 100 different oligonucleotides in discrete locations per square centimeter.

25. A solid support according to claim 20, wherein the support comprises at least about 1000 different oligonucleotides in discrete locations per square centimeter.
26. A solid support according to claim 20, wherein the support comprises at least about  
5 10,000 different oligonucleotides in discrete locations per square centimeter.
27. A computer system comprising:  
(a) a database containing information identifying the expression level in liver tissue of a set of genes comprising at least one gene in Table 1; and  
(b) a user interface to view the information.
- 10 28. A computer system of claim 27, wherein the database further comprises sequence information for the genes.
29. A computer system of claim 27, wherein the database further comprises information identifying the expression level for the genes in normal liver tissue.
30. A computer system of claim 27, wherein the database further comprises  
15 information identifying the expression level for the genes in tissue from a hepatic carcinoma.
31. A computer system of claim 30, wherein the hepatic carcinoma is from a patient with chronic hepatitis.
32. A computer system of claim 30, wherein the hepatic carcinoma is from a patient  
20 with cirrhosis.
33. A computer system of any of claims 27-32, further comprising records including descriptive information from an external database, which information correlates said genes to records in the external database.
34. A computer system of claim 33, wherein the external database is GenBank.
- 25 35. A method of using a computer system of any one of claims 27-34 to present information identifying the expression level in a tissue or cell of at least one gene in Table 1, comprising:

(a) comparing the expression level of at least one gene in Table 1 in the tissue or cell to the level of expression of the gene in the database.

36. A method of claim 35, wherein the expression level of at least two genes are compared.

5 37. A method of claim 35, wherein the expression level of at least five genes are compared.

38. A method of claim 35, wherein the expression level of at least ten genes are compared.

10 39. A method of claim 35, further comprising displaying the level of expression of at least one gene in the tissue or cell sample compared to the expression level in liver disease.

40. A method of claim 39, wherein the liver disease is hepatic carcinoma, chronic hepatitis or cirrhosis.

15 41. A therapeutic agent for slowing or halting the progression of liver cancer, wherein the agent is selected from the group consisting of the genes in Table 1, functional fragments of the genes in Table 1, proteins encoded by the genes in Table 1 and functional fragments of said proteins.

42. A method of treating a patient with liver cancer, comprising:

20 (a) administering to a patient with liver cancer a pharmaceutical composition comprising all or a portion of at least one gene in Table 1, or a protein encoded therein.

## SEQUENCE LISTING

<110> KOH, Sang Seok  
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<120> GENE EXPRESSION PROFILES IN LIVER DISEASE

<130> 44921-5109-WO

<150> US 60/341,815

<151> 2001-12-21

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<170> PatentIn Ver. 2.1

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&lt;210&gt; 2

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&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

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&lt;400&gt; 2

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 <223> n = a or c or g or t

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<220>  
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 <223> n = a or c or g or t

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 <213> Homo sapiens

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&lt;223&gt; Genbank Accession No. AI916306

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&lt;211&gt; 2834

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. X77777

&lt;400&gt; 9

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&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. X63359

&lt;400&gt; 10

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&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. Z48475

&lt;400&gt; 11

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<210> 21
<211> 447
<212> DNA
<213> Homo sapiens

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<223> n = a or c or g or t

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<210> 22
<211> 567
<212> DNA
<213> Homo sapiens

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<220>
<223> Genbank Accession No. AI052592

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<223> n = a or c or g or t

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<212> DNA
<213> Homo sapiens

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<220>
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<212> DNA
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<400> 24

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&lt;210&gt; 25

&lt;211&gt; 489

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AI424433

&lt;400&gt; 25

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&lt;210&gt; 26

&lt;211&gt; 6711

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. X52851

&lt;400&gt; 26

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&lt;210&gt; 27

&lt;211&gt; 542

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AF072812

&lt;400&gt; 27

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&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AF006043

&lt;400&gt; 28

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&lt;223&gt; Genbank Accession No. L04751

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&lt;213&gt; Homo sapiens

&lt;220&gt;

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&lt;400&gt; 35

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&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. U02390

&lt;400&gt; 37

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<213> Homo sapiens

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&lt;211&gt; 1324

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. D43642

&lt;400&gt; 40

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&lt;210&gt; 41

&lt;211&gt; 890

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. M91670

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&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AI478190

&lt;220&gt;

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&lt;222&gt; (1)..(457)

&lt;223&gt; n = a or c or g or t

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&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. U51007

&lt;400&gt; 43

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&lt;211&gt; 2385

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. X86809

&lt;400&gt; 55

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&lt;211&gt; 1689

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. U32680

&lt;400&gt; 56

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&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. D26600

&lt;400&gt; 57

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&lt;213&gt; Homo sapiens

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&lt;223&gt; Genbank Accession No. AL008726

&lt;400&gt; 58

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<211> 804

<212> DNA

<213> Homo sapiens.

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<223> Genbank Accession No. W22366

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<221> unsure

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<223> n = a or c or g or t

<400> 59

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&lt;210&gt; 60

&lt;211&gt; 691

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AI433892

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1)..(691)

&lt;223&gt; n = a or c or g or t

&lt;400&gt; 60

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&lt;210&gt; 61

&lt;211&gt; 537

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AA029831

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1)..(537)

&lt;223&gt; n = a or c or g or t

&lt;400&gt; 61

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&lt;210&gt; 62



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 <212> DNA  
 <213> Homo sapiens

<220>  
 <223> Genbank Accession No. AI279571

<220>  
 <221> unsure  
 <222> (1)..(592)  
 <223> n = a or c or g or t

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 <211> 460  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <223> Genbank Accession No. AI982714

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 <213> Homo sapiens

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&lt;210&gt; 65

&lt;211&gt; 737

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AI816034

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1)..(737)

&lt;223&gt; n = a or c or g or t

&lt;400&gt; 65

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 <212> DNA  
 <213> Homo sapiens

<220>  
 <223> Genbank Accession No. AI634570

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 <212> DNA  
 <213> Homo sapiens

<220>  
 <223> Genbank Accession No. AI023344

<220>  
 <221> unsure  
 <222> (1) .. (805)  
 <223> n = a or c or g or t

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<220>  
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&lt;210&gt; 69

&lt;211&gt; 549

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AA632300

&lt;400&gt; 69

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tagaaaaaca aaatggaaca tttattcgca actcaaatac tacgcatata cggtaagaga 60
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cgctcggga 549

```

&lt;210&gt; 70

&lt;211&gt; 460

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AI798743

&lt;400&gt; 70

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```

```

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gggagctgag aaaagtgtca ccagctgccg cttggcaaag aactcgtagc cgtcttctac 420
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<210> 71  
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 <212> DNA  
 <213> Homo sapiens

<220>  
 <223> Genbank Accession No. H98166

<220>  
 <221> unsure  
 <222> (1)..(626)  
 <223> n = a or c or g or t

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tgcaacagcc tctgagtgac ttgagccgca tgtgactggg gttctgttaa aagggcaggc 180
ncctccctcc tagccctgaa gccccaggaa cctgccttga aagacaagct ctctaatact 240
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 <212> DNA  
 <213> Homo sapiens

<220>  
 <223> Genbank Accession No. AA039699

<220>  
 <221> unsure  
 <222> (1)..(534)  
 <223> n = a or c or g or t

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tttgaangat attggaacag tttttttgat gacctttcag attctttctc tggaaactgaa 480
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<210> 73  
 <211> 981  
 <212> DNA  
 <213> Homo sapiens

<220>

&lt;223&gt; Genbank Accession No. D78132

&lt;400&gt; 73

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aataaaatag ctgcaaacct c 981

```

&lt;210&gt; 74

&lt;211&gt; 2263

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. U03985

&lt;400&gt; 74

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tcggtgggtc caggagcat tgcattcagt ttacctcaga gaaaatgggc tgggctttct 240
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&lt;210&gt; 75

&lt;211&gt; 1476

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. U16799

&lt;400&gt; 75

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&lt;210&gt; 76

&lt;211&gt; 825

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AI354351

&lt;400&gt; 76

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```

&lt;210&gt; 77

&lt;211&gt; 692

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. D26598

&lt;400&gt; 77

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```

&lt;210&gt; 78

&lt;211&gt; 1059

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. X95404

&lt;400&gt; 78

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```

&lt;210&gt; 79

&lt;211&gt; 3309

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. M15036

&lt;400&gt; 79

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&lt;210&gt; 83

&lt;211&gt; 377

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. W42636

&lt;400&gt; 83

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&lt;210&gt; 84

&lt;211&gt; 418

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. T66157

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1)..(418)

&lt;223&gt; n = a or c or g or t

&lt;400&gt; 84

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&lt;210&gt; 85

&lt;211&gt; 1811

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. M62831

&lt;400&gt; 85

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&lt;211&gt; 372

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. T98839

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1) .. (372)

&lt;223&gt; n = a or c or g or t

&lt;400&gt; 86

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&lt;211&gt; 924

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. U41344

&lt;400&gt; 87

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&lt;210&gt; 88

&lt;211&gt; 511

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AI765890

&lt;400&gt; 88

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&lt;210&gt; 89

&lt;211&gt; 318

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AL043089

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1) .. (318)

&lt;223&gt; n = a or c or g or t

&lt;400&gt; 89

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&lt;210&gt; 90

&lt;211&gt; 716

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. M11433

&lt;400&gt; 90

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&lt;210&gt; 91

&lt;211&gt; 2052

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. Y11307

&lt;400&gt; 91

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2052

&lt;210&gt; 92

&lt;211&gt; 748

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AI078144

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1)..(748)

&lt;223&gt; n = a or c or g or t

&lt;400&gt; 92

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&lt;210&gt; 93

&lt;211&gt; 602

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AA122235

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1)..(602)

&lt;223&gt; n = a or c or g or t

&lt;400&gt; 93

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ca                                     602
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&lt;210&gt; 94

&lt;211&gt; 674

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AI342377

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1)..(674)

&lt;223&gt; n = a or c or g or t

&lt;400&gt; 94

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angcccctcg tgcc
674

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&lt;210&gt; 95

&lt;211&gt; 1155

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. U86529

&lt;400&gt; 95

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&lt;210&gt; 96

&lt;211&gt; 391

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AI680350

&lt;220&gt;



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 <211> 1108  
 <212> DNA  
 <213> Homo sapiens

<220>  
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1108

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&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. M59371

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&lt;211&gt; 1247

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&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. AF040639

&lt;220&gt;

&lt;221&gt; unsure

&lt;222&gt; (1)..(1238)

&lt;223&gt; n = a or c or g or t

&lt;400&gt; 100

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&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Genbank Accession No. M12963

&lt;400&gt; 101

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&lt;213&gt; Homo sapiens

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&lt;223&gt; Genbank Accession No. K02402

&lt;400&gt; 102

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&lt;223&gt; Genbank Accession No. J03507

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&lt;220&gt;

&lt;223&gt; Genbank Accession No. K01383

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(57) Abstract:



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# PATENT COOPERATION TREATY

## PCT

### DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rule 13ter.1(c) and 39)

Applicant's or agent's file reference 44921-5109WO	<b>IMPORTANT DECLARATION</b>	Date of mailing (day/month/year) <b>04 JUN 2003</b>
International application No. PCT/US02/40718	International filing date (day/month/year) 20 December 2002 (20.12.2002)	(Earliest) Priority date (day/month/year) 21 December 2001 (21.12.2001)
International Patent Classification (IPC) or both national classification and IPC  IPC(7): A01N 37/18, 43/04; C12P 19/34; C12M 1/34; C12Q 1/68; G01N 33/00, 33/566 and US Cl.: 435/4, 6, 91.2, 287.2; 436/501, 94; 702/19; 514/2, 44		
Applicant GENE LOGIC, INC.		

This International Searching Authority hereby declares, according to Article 17(2)(a), that no international search report will be established on the international application for the reasons indicated below.

1. ☐ The subject matter of the international application relates to:
  - a. ☐ scientific theories.
  - b. ☐ mathematical theories
  - c. ☐ plant varieties.
  - d. ☐ animal varieties.
  - e. ☐ essential biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
  - f. ☐ schemes, rules or methods of doing business.
  - g. ☐ schemes, rules or methods of performing purely mental acts.
  - h. ☐ schemes, rules or methods of playing games.
  - i. ☐ methods for treatment of the human body by surgery or therapy.
  - j. ☐ methods for treatment of the animal body by surgery or therapy.
  - k. ☐ diagnostic methods practised on the human or animal body.
  - l. ☐ mere presentations of information.
  - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.
2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:
 

☐ the description
 ☒ the claims
 ☐ the drawings
3. ☐ The failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions prevents a meaningful search from being carried out:
 

☐ the written form has not been furnished or does not comply with the standard.
 ☐ the computer readable form has not been furnished or does not comply with the standard.

4. Further comments:  
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Form PCT/ISA/203 (July 1998)

International application No.  
PCT/US02/40718

4. Further comments:

The claimed methods, compositions, solid supports, and computer systems all require the use of the "at least one gene in Table 1." A review of Table 1, pages 35-42 of the disclosure, finds a listing of various nucleic acid "fragments" by name, SEQ ID NO., etc. A review of the associated Sequence Listing finds that none of the disclosed "genes" are in fact any gene. All of the sequences disclosed are nucleic acid in composition, thereby precluding any meaningful search for polypeptides or functional fragments thereof. The nucleic acid sequences disclosed are incapable of encoding any polypeptide as they (1) lack any promoter; (2) lack a start site; (3) lack a stop codon; and/or (4) are out of reading frame. While some claims are directed to methods and compositions of the genes or functional fragments thereof, the disclosure does not identify which, if any fragment, is useful or what the desired function is. Accordingly, no meaningful search can be established.

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